PC 122990

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR#: 0050845

February 17, 2006

MEMORANDUM:

SUBJECT: **MESOTRIONE** - Review of Special Toxicological Studies.

PC Code: 122990
DP Barcode Nos: D295934

From: Yudith Tesfaye,
Registration Action Branch I
Health Effects Division (7509C)

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Registration Action Branch I
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To: James Stone/Joanne Miller (RM 23)
Registration Division (7505C)

ACTION REQUESTED: Syngenta conducted special studies on Mesotrione in support of section 3 conditional registration. These mechanistic data intended for further refinement of the FQPA assessment. RD requested HED to review the data, prepare Data Evaluation Reports (DERs) and evaluate its impact on previous FQPA assessment.

I. CONCLUSIONS

The Registration Action Branch I (RAB 1) has reviewed these mechanistic data and provided the DERs (see the attachment) for mesotrione. The new information **did not** change the FQPA assessment prepared previous.

II. STUDIES REVIEWED

1. 28-Day Repeated-Dose Dietary Study in the Rat; Non-guideline

CITATION: Lees, D. (2000) Mesotrione: dynamic exposure (28 day duration in the rat). Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: CTL Study No.: XR6680, Syngenta No.: 1632-00, August 31, 2000. MRID 45651804. Unpublished

EXECUTIVE SUMMARY: The purpose of this study was to investigate the occurrence of ocular lesions, the activities of hepatic enzymes involved in tyrosine metabolism (i.e., tyrosine aminotransferase [TAT] and 4-hydroxyphenylpyruvate dioxygenase [HPPD]), and the levels of plasma tyrosine in Alpk:AP$_f$SD (Wistar-derived) male rats fed a variable concentration of mesotrione. In the main study, 20 rats/group were exposed to either a control diet or a diet containing a variable nominal concentration of mesotrione (0.3-100 ppm; mean = 2.387 mg/kg/day) in the diet for 28 consecutive days. The satellite study included a control group of 8 rats and a treated group of 44 rats (exposed to the same variable test diet as the main study). From the treated satellite group, 4 rats were sacrificed on Days 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, and 25, prior to changes in the dietary concentration. From the control satellite group, 4 rats were sacrificed on Days 3 and 15. The animals were examined for plasma tyrosine levels, TAT and HPPD activities, ocular lesions (main study only), and gross lesions at termination and systemic toxicity (i.e., effects on body weights, food consumption, clinical signs) throughout the study.

There were no treatment-related ocular lesions or clinical signs.

The activity of 4-hydroxyphenylpyruvate dioxygenase (HPPD) was decreased in the treated main study and satellite groups (0.017-0.218 uL O_2/min/mg protein) compared to controls (0.912-1.205 uL O_2/min/mg protein); this inhibition appeared to be dose-dependent. HPPD is involved in tyrosine catabolism. Thus, HPPD inhibition resulted in plasma tyrosine levels that were increased dose-dependently with increasing concentration of mesotrione in the diet. A consequence of increased plasma concentrations of tyrosine was an increase in the activity of tyrosine aminotransferase (TAT); TAT activity was increased in the treated main study and satellite groups (1.316-2.256 mmol HPPA/min/mg protein) compared to controls (1.078-1.991 mmol HPPA/min/mg protein).

Evidence of mild systemic toxicity included: decreased body weights in the treated main study animals (decr. 1-4%; p<=0.05), resulting in decreased overall body weight gains (decr. 8%) compared to controls; decreased food consumption in the treated main study animals on Days 1, 2, and 10 (decr. 4-6% p<=0.05); and pelvic dilatation of the kidney(s) in the treated main study (1/20 treated vs 0/20 controls) and satellite (1/44 treated vs 0/8 controls) groups.

This study is classified as an **acceptable/non-guideline** study in the rat.

2. Reproduction and Fertility Effects in the Rat; Non-guideline

CITATION: Williams, J. (2000) ZA1296: reproductive study in the pregnant rat in conjuction with tyrosine. Central toxicology laboratory, Cheshire, UK. Laboratory Study Id.: CTL Study Number 1356, Syngenta Number 857-97, September 27, 2000. MRID 45651805. Unpublished

EXECUTIVE SUMMARY: The purpose of this study was to evaluate the role of tyrosine in the ZA1296 induced reproductive effects by adding different dose levels of tyrosine in conjunction with ZA1296 in order to exacerbate any possible tyrosine related effects including decreased litter size, decreased pup survival, and bilateral hydronephrosis in the kidneys.

Treatment-related effects of the test substance, tyrosine, and the interaction of the test substance with different levels of tyrosine were examined in a 2 x 4 factorial study design. P females were fed one of 8 possible experimental diets containing the test substance (0 or 2500 ppm) and tyrosine (0, 0.5, 1.0, or 2.0%) from the day of arrival at the performing laboratory on gestation day (GD) 1 until study termination on lactation day (LD) 29. Maternal clinical observations, food consumption, and body weights were measured throughout the study. The dams were allowed to litter naturally; clinical observations, litter size, pup survival at post-natal day (PND) 22, and body weights were measured in the offspring. Plasma tyrosine levels were measured in the P dams on GD 3 and LD 29 (Study Day 51) and in the offspring on PND 29. Pelvic dilatation was examined in the offspring.

MATERNAL ANIMALS: One animal in the 0 ppm ZA1296/2% tyrosine group was found dead on GD 25, and one animal in the 2500 ppm ZA 1296/0% tyrosine group was terminated on GD 23 due to difficulties in parturition. All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 following rapid deterioration (i.e., hunched posture, piloerection, and severe eye lesions) during the first week of the study.

Dietary exposure to ZA1296, in the presence or absence of tyrosine, resulted in opacity in the eyes. Hunched posture was noted in 1/20 rat/group for 1-2 days in the groups treated with tyrosine in the absence of ZA1296. This clinical sign was also noted in the group exposed to ZA1296 alone (1/20 rats for 1 day) and tended to increase in incidence and frequency with increasing tyrosine concentration in the ZA1296 treated animals (1-5/20 rats for 1-12 days vs 0 controls).

Maternal body weights were decreased by 2-8% in the groups exposed to ZA1296 in the presence or absence of tyrosine during GD 4, 7, and 15 and in dams exposed to both ZA1296 and tyrosine generally throughout lactation. Body weight gain for the overall (LD 1-29) lactation period in the 2500 ppm ZA1296/1% tyrosine group was decreased by 54% compared to controls. Maternal food consumption was decreased by 11-14% in the dams fed 2500 ppm ZA1296 in the absence of tyrosine during Week 1 of gestation and Week 3 of lactation. The effect of ZA1296 on food consumption was more frequent and more pronounced with increasing concentration of tyrosine (0.5-1% tyrosine), with decreases of 13-23% during Weeks 1 and 2 of gestation and decreases of 17-53% throughout lactation.

In the absence of ZA1296, plasma tyrosine levels were increased over controls on GD 3 in the 2%

tyrosine dams (293 nmol/mL treated vs 182 nmol/mL controls) and on LD 29 in the 1% and 2% tyrosine dams (146 nmol/mL each treated vs 109 nmol/mL controls). In the presence of ZA1296, the increases in plasma tyrosine levels were more pronounced, irrespective of tyrosine levels in the diet (2010-3475 nmol/mL treated vs 109-182 nmol/mL controls).

OFFSPRING: Viability at birth and survival during the post-natal period were affected by treatment with ZA1296 and/or tyrosine. The proportion of pups born live was decreased in the 2% tyrosine group and in the ZA1296 groups with 0.5 and 1% tyrosine. Additionally in the 1% tyrosine/2500 ppm ZA1296 group, decreases were observed in the percentage of pups born live (86.2% treated vs 97.8% controls) and in the proportion of litters with all pups born live (6/18 treated vs 16/19 controls). The following offspring survival parameters were decreased ($p<=0.01$): (i) litter size throughout the post-natal period after PND 1 in the ZA1296 groups with 0.5% tyrosine (decr. 40-58%) and 1% tyrosine (decr. 74-84%); (ii) proportion of pups surviving to PND 22 in the ZA1296 treated groups in the presence or absence of tyrosine and in the >=1% tyrosine groups not fed ZA1296; (iii) percentage of pups surviving to PND 22 in the ZA1296 groups with 0.5 and 1% tyrosine (31.6-59.3% treated vs 93.1% controls); and (iv) proportion of litters with 100% survival in the 1% tyrosine/2500 ppm ZA1296 group (0/18 treated vs 12/19 controls). The decreases in offspring survival were reflected in decreased litter weights in the ZA1296 group with 0.5% tyrosine generally after PND 1 (decr. 20-36%) and in the ZA1296 group with 1% tyrosine throughout the post-natal period (decr. 18-66%); these decreases became more severe with time. Pup body weights were comparable to controls in both sexes throughout the post-natal period.

Clinical signs of toxicity were limited to offspring from dams fed ZA1296. Cloudy and/or opaque eyes were noted in offspring exposed to ZA1296 in the presence or absence of tyrosine (2-10 pups; 9-70 observations) vs 0 controls. Shaking and piloerection were observed in the 1% tyrosine/2500 ppm ZA1296 group (2 pups; 3 observations).

In offspring not exposed to ZA1296, plasma tyrosine levels were increased in the males at all tyrosine concentrations and in the females at 1 and 2% tyrosine (163-227 nmol/mL treated vs 127-128 nmol/mL controls). Tyrosine levels were increased much more severely in offspring exposed to ZA1296, irrespective of tyrosine concentration (1942-2169 nmol/mL treated vs 127-128 nmol/mL controls).

Incidences of pelvic dilatation in the kidneys were observed in the male and female pups treated with ZA1296 in the presence or absence of tyrosine (33-52% vs 0-10% controls). There were no treatment-related effects of tyrosine alone on pelvic dilatation in the kidneys in either sex but doses of tyrosine alone were much lower than when combined with ZA1296.

In conclusion, this study was successful in demonstrating the effects of ZA1296, tyrosine, and the combination of ZA1296 and tyrosine on the parameters observed in the previous reproductive toxicity study including decreased pup survival and litter size and increased incidences of pelvic dilation of the kidneys.

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The statistical analyses conducted were not the most appropriate for the study design. This study employed a 2 x 4 factorial design, in which the effects of two levels of ZA1296 and 4 levels of tyrosine were tested in all possible combinations for a total of eight treatments. The Sponsor compared all of the groups using one-way ANOVA or ANCOVA, followed by pair-wise comparisons of each treated group with the control. A more powerful and appropriate statistical test would have been a two-factor test, testing the effects of ZA1296, tyrosine, and the interaction of ZA1296 and tyrosine (ZA1296*tyrosine). However, this deficiency does not affect the conclusions of this DER or the acceptability of the study.

3. Non-guideline Study; Investigation of Liver and Kidney Enzyme Parameters - Mice.

CITATION: Williams, J. (2001) Investigation of liver and kidney enzyme parameters in control mouse pups from new born to age 42 days. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study Id.: CTL Study No. RM0801, Syngenta No. 1251-98, January 23, 2001. MRID 45651807. Unpublished.

EXECUTIVE SUMMARY: In a non-guideline study (MRID 45651807), 54 untreated pregnant Alpk:AP$_f$CD-1 female mice were allowed to litter normally. Six females and their litters were killed on postnatal days (PND) 1, 2, 3, 4, 5, and 8; three females and their litters were killed on PND 12, 15, 22, 29, 35, and 42. These animals were exsanguinated, and the liver and kidneys were removed. The levels of plasma tyrosine, liver and kidney tyrosine aminotransferase (TAT) activity, and liver and kidney 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity were determined. The purpose of this study was to provide a database on the basal levels of plasma tyrosine and liver and kidney enzymes (TAT and HPPD) involved in tyrosine catabolism in control maternal mice and mouse pups up to 42 days of age.

Maternal plasma tyrosine levels appeared variable with standard deviations reaching $\pm$ 26.2 on PND 15. They appeared to rise from PND 1 to PND 3, although variations continued to be large expecially as noted on PND 29. Maternal liver TAT activity peaked on PND 2, and on PND 3 began to decrease with some level of stability by PND 29. Maternal kidney TAT activity also was variable. It peaked on PND 3, fell through PND 15, then rose through PND 42. Liver HPPD activity appeared less variable throughout the study. Kidney HPPD activity was much lower than in the liver and but still appeared variable over time. Kidney TAT activity was approximately 8-fold lower than that observed in liver, while kidney HPPD activity was substantially (approximately 44-fold) lower than in liver.

Pup plasma tyrosine levels rose steadily from birth, peaking on PND 12 for both sexes. However, values were also variable with plasma tyrosine levels then falling through PND 29 and then rising somewhat again near the end of the sampling period on day 42 in both sexes. In general, pup plasma tyrosine levels were higher for PND 1-42 than that observed in maternal females. Liver TAT activity appeared to fluctuate greatly throughout the study, with males and females exhibiting low values on PND 12 and high values on PND 29. In general, liver TAT activity in both sexes was lower than that observed in adult females although there was some overlap of values. Kidney TAT activity was also

quite variable. It rose from a low on PND 1-2, approached adult female levels by PND 4, and fluctuated for the remainder of the study. Liver HPPD activity rose from a low on PND 1-2, approached adult female levels by PND 22, and fluctuated widely for the remainder of the study. Kidney HPPD activity rose from a low on PND 1, approached adult female levels by PND 4, and fluctuated widely for the remainder of the study. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities.

In summary, in neonatal pups, plasma tyrosine levels rose steadily from birth until PND 12 and appeared to become lower and more variable by the end of the sampling period. In both sexes, plasma tyrosine levels appeared slightly higher at PND 42 than that observed in maternal females. In general, liver TAT activity in both sexes was lower than that observed in adult females. Kidney TAT activity approached adult female levels by PND 4. Liver HPPD activity approached adult female levels by PND 22, while kidney HPPD activity approached adult female levels by PND 4. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities. In addition, results during many sampling periods varied widely and it is unclear whether this is associated with biochemical analyses methodology problems, small sample size or whether the values actually varied within the test population at the intervals sampled. Replicate analyses would be necessary to confirm results. For the above listed reasons, it is unclear whether useful background data have been obtained in this study.

The submitted study is classified as **acceptable/non-guideline**.

4. Non-guideline Study; Prenatal Developmental Toxicity Study - Mice.

CITATION: Moxon, M. (2001) Prenatal development toxicity: method development study in the mouse to achieve compliance with EPA guideline. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RM0811, Syngenta No. 1254-98, April 10, 2001. MRID 45651810. Unpublished.

Moxon, M..E. (2001) ZA1296: Dose range finding study in mice. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RM0799, Syngenta No. 1252-98, April 10, 2001. MRID 45651808. Unpublished.

EXECUTIVE SUMMARY:

In a non-guideline developmental toxicity study (MRID 45651810), water was administered daily by oral gavage at a dose volume of 1 mL/100 g body weight to 50 Alpk:AP$_f$CD-1 female mice on gestation days (GD) 5 through 18. All mice were sacrificed on GD 19; their fetuses were removed

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by cesarean and examined. The objective of this study was to establish standard operating procedures for the staining and evaluation of fetal bone and cartilage, and to provide background control data to support future developmental toxicity studies in the mouse.

Ten of the 50 mice did not survive to scheduled termination. One mouse was killed following premature delivery on GD 17, and one mouse died during observation on GD 6. The deaths of the other 8 mice were attributed to poor dosing technique. Two mice were found dead, one each on GD 6 and 13. Six mice were sacrificed due to clinical signs; one on GD 6, two on GD 10, one on GD 11, and two on GD 18. Clinical signs included altered breathing pattern, hunched posture, piloerection, subdued behavior, shaking, closed eyes, cold to touch, and pallor. Food consumption was observed to increase slightly over the treatment period. The mice were observed to almost double their body weight by GD 19; however, gravid uterus weight accounted for the majority of the increase. *Post Mortem* macroscopic findings were only reported for 10 of the 50 mice on study (perhaps only those that died on study). This would significantly limit the assessment of potential dosing errors during the study to animals that survived to term. A few findings were noted for mice that survived to term but it does not appear that complete necropsies were performed for 80% of animals on test. One mouse that survived to sacrifice was observed to have a mass in the thoracic cavity that was adherent to the heart, lungs, thoracic wall, and diaphragm, an enlarged spleen, and the liver was adherent to the diaphragm. However, these findings were also considered by the investigators as due to poor dosing technique. In mice that died *in extremis* or were killed prior to study termination, the most common findings, indicative of dosing errors, were adhesions of the lungs, perforated esophagus, excess watery fluid in the thoracic cavity, and adhesions in the thoracic cavity. Since 6 of the 8 deaths attributed to poor dosing technique occurred on or prior to GD 13, the dosing technique was considered by the investigators to have improved to some minimal extent during the latter portion of the study.

The overall pregnancy rate for the study was 74%. The pregnancy rates for mice delivered to the performing laboratory on GD 1, 2, or 3 were 90.5%, 75.0%, and 46.2%, respectively. Due to this substantial decrease in pregnancy rate, it was concluded by the investigators that the optimal time for shipment of pregnant mice was GD 1. However, these differences could have easily been due to numerous other factors including variability in rates at shipment and stress associated with dosing error. In addition, it is clear that 13 of 50 females were not pregnant at term and this left only 37 of 50 animals for litter assessment.

One dam was killed following a premature delivery, and complete resorption was observed in another dam that survived to termination and at necropsy was found to have signs of misdosing. Three fetuses were found to have major external defects (extra digits of the forepaw, malrotated hindlimb, and cleft palate). One fetus was found to have a major visceral defect (missing aortic arch). Six fetuses were found to have major skeletal defects. Four of these fetuses came from one litter of 20 fetuses; all exhibited multiple shortened bones in the fore and hindlimbs, including bilateral curved and shortened radius, bilateral shortened ulna, humerus, femur, fibula, tibia, scapula, and ilium. Fused premaxillae and extra metacarpals and misshapen metacarpals were also noted. The remaining 2 fetuses came from a single litter; one fetus was observed to have thoracic arches

7 and 8 fused and cartilage fused between arches 5 and 6, while the other was noted to have multiple minor defects that were collectively classified as a malformation. Four fetuses were found to have minor external defects (slight flexion of the hindlimb or kink of the tail). Nineteen fetuses were found to have minor visceral defects (discolored thymus or umbilical artery left of the bladder). Numerous skeletal variants and minor defects were observed. The variants occurring with the greatest incidence (>=10% of fetuses) were: (i) centrum 3, 4, and 5 not ossified; (ii) small holes in sternebrae 6; (iii) xiphoid cartilage cleft; (iv) 7th cervical rib shortened; and (v) hindpaw calcaneum ossified. The minor defects occurring with the greatest incidence (>=10% of fetuses) were: (i) small holes in supraoccipital bones; (ii) slight incomplete ossification of the parietal bones; (iii) sternebrae 4 incompletely cleft; (iv) sternebrae 5 and 6 hemicenters incompletely fused; and (v) 7th cervical rib long. Other variants and minor defects were noted, but occurred in less than 10% of fetuses. The mean (±SD) score for *manus* ossification was 4.20±0.68, while the mean score for *pes* ossification was 4.14±1.03.

Apparently, no effort was made by the investigators to compare incidences of malformations and variations observed in this study with background levels reported by other investigators in the literature. This would be essential in order to validate this laboratory's methodology especially considering the rarity of some of the findings reported. In addition, the issue of dosing stress (relative to poor dosing technique) was not even considered by the investigators relative to the findings observed. As well, litter data generated do not appear useful for historical control purposes due to the confounding influence of dosing error and unnecessary dosing stress observed in this study.

In conclusion, neither the ability of the testing facility to use the mouse as a test model nor its ability to successfully generate useful historical control data was demonstrated in this study.

The submitted study is classified as **unacceptable/non-guideline**.

5. Non-guideline Study: Investigation of Liver and Kidney Enzyme Parameters - Rats.

CITATION: Moxon, M.E. (2000) Investigation of liver and kidney enzyme parameters in control rat pups from new born to age 42 days. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RR0798, Syngenta No. 1253-98, November 16, 2000. MRID 45651809. Unpublished.

EXECUTIVE SUMMARY: In a non-guideline study (MRID 45651809), 36 untreated time-mated pregnant Alpk:AP$_f$SD female rats were received on gestation day (GD) 1 and allowed to litter normally. Three females and their litters were killed on postnatal days (PND) 1, 2, 3, 4, 5, 8, 12, 15, 22, 29, 35, and 42. These animals were exsanguinated, and the liver and kidneys were removed. The levels of plasma tyrosine, and liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities were determined. The purpose of this study was to provide a database on the basal levels of plasma tyrosine and liver and kidney TAT and HPPD in control rat pups up to 42 days of age.

Maternal plasma tyrosine levels appeared to rise from 64.6 nmol/mL on PND 1 to 105.6 nmol/mL on PND 29. Thereafter, the levels reduced to the end of the sampling period on day 42. Maternal liver TAT activity fluctuated over the course of the sampling period without any apparent pattern over time. A low value was observed on PND 4 (0.211 nmol HPPA/min/mg protein) perhaps due to experimental error. Maternal kidney TAT levels were approximately 4-fold lower than liver levels. Kidney TAT activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time.

Maternal Liver HPPD activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time. Kidney HPPD activity also varied over the sampling period but generally was half to two-thirds of the liver levels. In addition, although kidney HPPD activity appeared quite variable, the range of fluctuation was smaller than observed in the liver.

Pup plasma tyrosine levels rose from birth, peaking on PND 8-12 for both sexes. Plasma tyrosine levels then abruptly fell on PND 22 to approximately the same level as observed in adult females. Pup plasma levels remained lower but variable over the remainder of the sampling period.
Liver TAT activity appeared to fluctuate throughout the study, with male and female offspring (4.943-5.444 nmol HPPA/min/mg protein vs 3.347 nmol HPPA/min/mg protein) having approximately the same activity as adult females. Kidney TAT levels were approximately 7-fold lower than liver levels. Kidney TAT activity also appeared to fluctuate throughout the study, with male and female offspring (0.696-0.798 nmol HPPA/min/mg protein vs 0.797 nmol HPPA/min/mg protein) having approximately the same range of activity as in adult females.

Pup liver HPPD activity (in both sexes) generally rose from a low on PND 1 (0.484-0.625 µL O_2/min/mg protein), and after significant fluctuation increased markedly after PND 15 exceeding adult female levels by PND 22. Thereafter, levels fluctuated (although at increased levels) for the remainder of the study. Kidney HPPD levels were approximately 3-fold lower than liver levels. Kidney HPPD activity rose from a low on PND 1 (0.187-0.194 µL O_2/min/mg protein) and approached adult female levels by PND 22. Activity fluctuated for the entire sampling period of the study.No apparent differences between sexes were observed up to PND 42 in plasma tyrosine levels or liver and kidney TAT and HPPD activities.

It is unclear how (or if) problems with analytical methodology might have contributed to the variability of activity for tyrosine, liver and kidney TAT and HPPD activities in this study. As well, small sample size may have contributed to the variability of activity observed at different sampling intervals. In addition, without replicate analyses, it is difficult to assess the utility of these data as background levels for this test species.

The submitted study is classified as **acceptable/non-guideline**.

6. Non-guideline Study; Investigation of the Effects of ZA1296 and Tyrosine on Developmental Toxicity in the Rabbit.

CITATION: Moxon, M.E. (2000) Investigation of the effects of ZA1296 and tyrosine on developmental toxicity in the rabbit. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study Id.: CTL Study No. RB0802, Syngenta No. 1281-99, April 13, 2000. MRID 45651812. Unpublished.

EXECUTIVE SUMMARY: In a non-guideline developmental toxicity study (MRID 45651812), ZA1296 (Mesotrione; Lot/batch # P17; 96.8% a.i.) was administered in water daily by oral gavage at a dose volume of 10 mL/kg body weight to 20 New Zealand White female rabbits/group at dose levels of 0 or 500 mg/kg on gestation days (GD) 8 through 20. Additionally, rabbits were fed either diet containing 1% tyrosine or control diet during this period, such that there were 4 experimental groups total (2 x 2 factorial design): i) water gavage + control diet (group 1); ii) water gavage + 1% tyrosine diet (group 2); iii) 500 mg/kg ZA 1296 gavage + control diet (group 3); and iv) 500 mg/kg ZA1296 gavage + 1% tyrosine diet (group 4). All does were sacrificed on GD 30, and their fetuses were removed and examined. Maternal plasma tyrosine levels and liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities were determined. The objective of this study was to manipulate maternal plasma tyrosine levels in order to investigate the effects of tyrosinaemia on fetal skeletal ossification, and to determine if treatment with ZA1296 caused increased incidence of abortion.

Maternal Toxicity:

There were no effects of treatment on maternal survival, clinical signs, ophthalmoscopic examination, or gross pathology reported. Maternal body weights (adjusted for initial weight) were decreased ($p \leq 0.05$) in the group 4 (500 mg/kg ZA1296 + 1% tyrosine) does during GD 14-21 (↓1-3%), and remained decreased (not significant) through GD 30 (↓2%). Overall (GD 4-30) body weight gains were also decreased in the group 4 does (↓7%) compared to controls. However, in the rabbit both the small body weight and body weight gain changes as observed in this study are considered normal variation, minor and not biologically significant and do not constitute maternal toxicity. As well, food consumption was decreased ($p \leq 0.05$) in the group 4 does by 27% on GD 8-11 and continued throughout the remainder of the dosing period (GD 11-21), although without statistical significance (↓8-22%). Additionally, the group 3 (500 mg/kg ZA1296 only) does were observed to have decreased food consumption throughout dosing (GD 8-21; ↓7-13%; not significant). In both cases, food consumption returned to control levels by GD 24-27. Rabbits are notorious for spillage of feed and differences like these in the absence of clear body weight changes are not considered biologically relevant.

Plasma tyrosine levels were increased ($p \leq 0.01$) in all groups in a step-wise fashion, with the greatest increases occurring in the group 4 does (↑284-1773%). Levels peaked in all groups at 12 hours after treatment with ZA1296, and returned to maintenance levels at 24 hours post-dosing. Kidney TAT activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓42-58%) compared to controls. Liver HPPD activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓54-57%). Kidney HPPD activity was decreased ($p \leq 0.01$) in the group 3 and 4 does (↓69-75%). No clinical signs or pathology were associated with these findings. Therefore, clear indications of maternal toxicity

were not observed in this study.

Developmental Toxicity:

One group 4 doe was killed on GD 22 following the abortion of several fetuses. This animal had demonstrated negligible food consumption from GD 17 and a loss of body weight from GD 19. Necropsy revealed a flaccid heart with pale areas on the ventricles; however, this finding was not considered to be treatment-related. No effects of treatment were observed on numbers of live fetuses, resorptions (early or late) or post-implantation loss.

No effects on fetal body weight were apparent. Increased ($p \leq 0.05$) incidences of the following skeletal defects were noted: incomplete ossification of the odontoid of the cervical centra in groups 3 (13.6% fetuses; 38.9% litters) and 4 (14.5% fetuses; 52.9% litters), and incompletely ossified pubis in group 4 (6.9% fetuses; 47.1% litters). The proportion of animals having a manus score of 3 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a manus score of 4. Similarly, the proportion of animals having a pes score of 1 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a pes score of 2.

Increased incidence ($p \leq 0.05$) of extra vessel(s) arising from the aortic arch was observed in groups 3 (4.8% fetuses; 27.8% litters) and 4 (6.9% fetuses; 29.4% litters). Enlarged ventricle and reduced ventricle of the heart were observed in group 2 (1% tyrosine only) fetuses (0.8% fetuses; 7.1% litters) and group 4 (1.4% fetuses; 11.8% litters) fetuses. Increased ($p \leq 0.05$) incidences of the following findings were observed: long thoracolumbar rib 13 in groups 2 (35.2% fetuses; 92.9% litters), 3 (73.5% fetuses; 88.9% controls), and 4 (92.4% fetuses; 100% litters), and 27 pre-pelvic bilateral vertebrae in groups 3 (49.7% fetuses; 88.9% litters) and 4 (86.2% fetuses; 100% litters). Extreme constriction of the pulmonary artery was noted in groups 3 (0.7% fetuses; 5.6% litters) and 4 (1.4% fetuses; 11.8% litters), and extreme dilation of the aorta was observed in groups 2 (0.8% fetuses; 7.1% litters), 3 (1.4% fetuses; 11.1% litters), and 4 (2.1% fetuses; 11.8% litters).

This is not a guideline study and due to study design, no dose response assessment was possible; a NOAEL and LOAEL were not determined. **In conclusion, in the absence of maternal toxicity, developmental toxicity was observed in this study with ZA1296 (Mesotrione, 96.8% a.i.) at a dose level of 500mg/kg (gavage) with either 1% or 0% tyrosine mixed in the diet.**

The submitted study is classified as **acceptable/non-guideline**.

DERs attached:

45651804.der
45651805.der
45651810.der

45651812.der
45651809.der
45651807.der

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PC 123916
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DATA EVALUATION RECORD

MESOTRIONE (ZA1296)

Study Type: Non-guideline, 28-day Repeated-Dose Dietary Study in Rats

Work Assignment No. 1-01-16 A (MRID 45651804)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: 28-Day Repeated-Dose Dietary Study in the Rat; Non-guideline

PC CODE: 122990

DP BARCODE: D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): Mesotrione technical (ZA1296; 97.6% a.i.)

SYNONYMS: 2-{4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Lees, D. (2000) Mesotrione: dynamic exposure (28 day duration in the rat). Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: CTL Study No.: XR6680, Syngenta No.: 1632-00, August 31, 2000. MRID 45651804. Unpublished

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC

EXECUTIVE SUMMARY: The purpose of this study was to investigate the occurrence of ocular lesions, the activities of hepatic enzymes involved in tyrosine metabolism (i.e., tyrosine aminotransferase [TAT] and 4-hydroxyphenylpyruvate dioxygenase [HPPD]), and the levels of plasma tyrosine in Alpk:AP$_f$SD (Wistar-derived) male rats fed a variable concentration of mesotrione. In the main study, 20 rats/group were exposed to either a control diet or a diet containing a variable nominal concentration of mesotrione (0.3-100 ppm; mean = 2.387 mg/kg/day) in the diet for 28 consecutive days. The satellite study included a control group of 8 rats and a treated group of 44 rats (exposed to the same variable test diet as the main study). From the treated satellite group, 4 rats were sacrificed on Days 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, and 25, prior to changes in the dietary concentration. From the control satellite group, 4 rats were sacrificed on Days 3 and 15. The animals were examined for plasma tyrosine levels, TAT and HPPD activities, ocular lesions (main study only), and gross lesions at termination and systemic toxicity (i.e., effects on body weights, food consumption, clinical signs) throughout the study.

There were no treatment-related ocular lesions or clinical signs.

The activity of 4-hydroxyphenylpyruvate dioxygenase (HPPD) was decreased in the treated main study and satellite groups (0.017-0.218 μL O_2/min/mg protein) compared to controls (0.912-

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1.205 uL O_2/min/mg protein); this inhibition appeared to be dose-dependent. HPPD is involved in tyrosine catabolism. Thus, HPPD inhibition resulted in plasma tyrosine levels that were increased dose-dependently with increasing concentration of mesotrione in the diet. A consequence of increased plasma concentrations of tyrosine was an increase in the activity of tyrosine aminotransferase (TAT); TAT activity was increased in the treated main study and satellite groups (1.316-2.256 mmol HPPA/min/mg protein) compared to controls (1.078-1.991 mmol HPPA/min/mg protein).

Evidence of mild systemic toxicity included: decreased body weights in the treated main study animals (decr. 1-4%; $p<=0.05$), resulting in decreased overall body weight gains (decr. 8%) compared to controls; decreased food consumption in the treated main study animals on Days 1, 2, and 10 (decr. 4-6% $p<=0.05$); and pelvic dilatation of the kidney(s) in the treated main study (1/20 treated vs 0/20 controls) and satellite (1/44 treated vs 0/8 controls) groups.

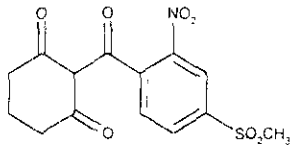
This study is classified as an **acceptable/non-guideline** study in the rat.

COMPLIANCE - Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Mesotrione technical (ZA1296)

Description:	Beige solid
Lot/Batch #:	P22
Purity:	97.6% a.i.
Compound Stability:	The test substance was stable in the diet for up to 16 days at -20°C.
CAS #:	104206-82-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Alpk:AP$_f$SD
Age at study initiation:	Approximately 6 weeks
Wt. at study initiation:	165-209 g
Source:	Rodent Breeding Unit, AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
Housing:	Four per cage
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22±3°C **Humidity:** 30-70% **Air changes:** ≥15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least 7 days

B. STUDY DESIGN

1. In life dates: Start: February 29, 2000 End: April 4, 2000

2. Purpose: The purpose of this study was to investigate the occurrence of ocular lesions, the activities of hepatic enzymes involved in tyrosine metabolism (i.e., tyrosine aminotransferase [TAT] and 4-hydroxyphenylpyruvate dioxygenase [HPPD]), and the levels of plasma tyrosine in rats fed a variable concentration of mesotrione (0.3-100 ppm) in the diet for 28 consecutive days.

3. Study design and schedule: In the main study, 20 male Alpk:AP$_f$SD (Wistar-derived) rats/group were exposed to either a control diet or a diet containing a variable nominal concentration of mesotrione (0.3-100 ppm) for 28 days. These animals were examined for plasma tyrosine levels, TAT and HPPD activities, and ocular lesions at termination (Day 29) and systemic toxicity (i.e., effects on body weights, food consumption, clinical signs) throughout the study. Additionally, a satellite control group of 8 rats and a satellite treated group of 44 rats were included for measurement of plasma tyrosine levels and TAT and HPPD activities at intervals

during the study. From the treated satellite group, 4 rats were sacrificed on Days 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, and 25, prior to changes in the dietary concentration. From the control satellite group, 4 rats were sacrificed on Days 3 and 15. Individual body weight and food consumption data were reported for the satellite animals.

3. Animal assignment: Animals were randomly assigned (blocked by location within the experimental array) to the test groups shown in Table 1. Detailed information on the study schedule and design, including the temporal variation in the nominal dose and the corresponding daily achieved dose, is included as an Appendix in this DER.

TABLE 1. Animal assignment [a]

Study	Test group	Nominal conc. in diet (ppm)	Achieved dose (mg/kg/day)	# Males
Main	Treated	0.3-100	0.025-13.029	20
	Control	0	0	20
Satellite [b]	Treated	0.3-100	0.025-13.029	44
	Control	0	0	8

a Data obtained from Study Report, pages 15, 16, 18, and 59.

b In the treated satellite group, 4 rats were sacrificed on Days 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, and 25, prior to changes in the dietary concentration. In the control satellite group, 4 rats were sacrificed on Days 3 and 15.

4. Dose selection rationale: It was stated that the dose levels were selected based on results from other feeding studies conducted in the performing laboratory using this strain of rats; however, no further information was provided.

5. Diet preparation and analysis: Premixes were prepared by mixing the appropriate amount of test substance (corrected for purity) with 50 g of diet (for the 0.3, 1, 3, 10, and 30 ppm diets) or 500 g diet (for the 0 or 100 ppm diets). These premixes were added to additional diet to achieve a 15 kg batch of each final concentration. The test diets were stored at approximately -20°C, and were defrosted for at least one hour before feeding. Samples of the test diets were taken immediately after preparation and stored at -20°C for possible analysis. However, concentration analyses were not reported. In a previously reviewed combined chronic toxicity/oncogenicity study (MRIDs 44505035 and 44505036), homogeneity (top, middle, bottom) was determined by analyzing samples from the 2500, 2.5, and 1 ppm dose formulations. Stability was determined at concentrations of 7000 and 1 ppm over a period of up to 16 days at room temperature and up to 40 days at -20°C.

Results

Homogeneity: 75-106%

Stability: 84.8-107.4% at room temperature.
87.5-114.6% at -20°C

Concentration: Not reported

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Although concentration analyses were not reported for test diet samples taken during this study, the analytical data indicated that the homogeneity and stability of the test diets were acceptable.

6. Statistics: The following statistical analyses were applied to the data:

Parameter	Statistical test
Body weights	Analysis of covariance (ANCOVA) on initial (Day 1) body weight
Food consumption	Analysis of variance (ANOVA)

Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. Plasma tyrosine levels, tyrosine aminotransferase activity, and 4-hydroxyphenylpyruvate dioxygenase activity were not analyzed statistically.

C. METHODS

1. Observations

a. Cageside Observations: Animals were inspected daily for clinical signs of toxicity and mortality.

b. Clinical Examinations: Clinical examinations were conducted prior to study initiation, daily for the first week of the study, and weekly thereafter.

2. Body weight: Each animal was weighed prior to study initiation, daily for the first week of the study, and weekly thereafter.

3. Food consumption and compound intake: Food consumption was recorded daily throughout the study for each cage of rats and was reported (g/animal/day) daily for the first week and thereafter at weekly intervals. Compound intake (mg/kg bw/day) values were calculated daily throughout the study using the food consumption and body weight data and the nominal dose.

4. Ophthalmoscopic examination: The eyes of all main study animals were examined prior to treatment and on Day 27. The examination was carried out using an indirect ophthalmoscope after instillation of 0.5% (v/v) tropicamide into the eyes to dilate the pupils.

5. Plasma tyrosine levels: Plasma tyrosine levels were measured in blood samples collected by cardiac puncture from all rats (main and satellite groups) at scheduled termination.

6. Sacrifice and pathology: At scheduled termination, all animals (main and satellite groups) were subjected to gross pathological examination, and liver cytosol samples were analyzed for tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities. It was stated that any abnormal tissues observed at necropsy were stored in an appropriate fixative; however, microscopic examinations were not performed.

II. RESULTS

1. Observations: There was a slightly increased incidence of tail damage, scabs on the tail, and scaly tail in the main study treated group compared to controls (Table 2). However, it is unlikely that these effects were due to treatment because similar clinical observations were not consistently observed in the treated satellite group. No other clinical signs could be attributed to treatment.

TABLE 2. Selected clinical observations [#animals (#observations)] in rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Parameter	Main study groups		Satellite groups	
	0 ppm	0.3-100 ppm	0 ppm	0.3-100 ppm
Scabs - coded by area 10	0 (0)	2 (9)	0 (0)	1 (6)
Scabs - coded by area 20	0 (0)	1 (5)	0 (0)	0 (0)
Tail - damaged	0 (0)	3 (12)	0 (0)	0 (0)
scaly	2 (4)	5 (22)	0 (0)	0 (0)

a Data were obtained from Table 1 on page 28 in the study report.

2. Body weight: Body weights were decreased (↓1-4%; $p \leq 0.05$) in the treated main study animals intermittently during the first week and each week thereafter, resulting in decreased (↓8%) overall body weight gains (Table 3). There were no other treatment-related differences in body weights. Only individual body weight data were provided for the satellite groups.

TABLE 3. Selected mean (± SD) body weights and overall body weight gains (g) in main study rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Study day	Dose (ppm)	
	0	0.3-100
1 [b]	190.0 ± 10.2	191.2 ± 8.6
2	197.4	195.2** (↓1)
3	205.0	203.2* (↓1)
7	235.6	232.8* (↓1)
15	296.7	290.4* (↓2)
22	334.9	325.2* (↓3)
29	365.6	351.5* (↓4)
Weight gain (Days 1-29) [c]	174.7	161.2 (↓8)

a Data were obtained from Table 3 on pages 31-32 in the study report; n = 20. Percent difference from controls, calculated by the reviewers, is included in parentheses.

b Body weights after Day 1 are adjusted based on the body weight on Day 1 as a covariate.

c Overall (Days 1-29) body weight gains were calculated by the reviewers as the difference in unadjusted group mean body weights on Days 1 and 29.

3. Food consumption and compound intake: Food consumption was decreased (↓4-6%; $p \leq 0.05$) in the treated main study animals on Days 1, 2, and 10 (Table 4). There were no other treatment-related differences in food consumption. Only individual food consumption data were provided for the satellite groups. Daily test substance intake values are reported in Table 1. The overall (Days 1-28) mean achieved dose for the treated animals was 2.387 mg/kg/day.

TABLE 4. Selected mean (± SD) food consumption (g/animal/day) in main study rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Study day	Dose (ppm)	
	0	0.3-100
1	26.8 ± 0.7	25.2 ± 0.7* (↓6)
2	26.2 ± 0.2	25.2 ± 0.5* (↓4)
10	32.2 ± 0.7	30.7 ± 0.8** (↓5)

a Data were obtained from Table 4 on pages 33-34 in the study report; n = 5 cages. Percent difference from controls, calculated by the reviewers, is included in parentheses.

4. Ophthalmoscopic examination: No treatment-related effects were observed in the eyes of treated animals in the main study.

5. Plasma tyrosine levels: Plasma tyrosine levels increased dose-dependently with increasing concentration of mesotrione in the diet (Table 5).

TABLE 5. Mean (± SD) plasma tyrosine levels (nmol/mL) in male rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Nominal concentration in diet (ppm)	Plasma tyrosine (nmol/mL)
0	107.8 ± 7.9
0.3	171.2 ± 9.4
1	331.2 ± 42.1
3	705.5 ± 169.4
10	1467.1 ± 68.0
30	1667.3 ± 123.5
100	1937.1 ± 10.6

a Data were calculated by the reviewers from Table 5 on page 37 in the study report by averaging the means from rats in the main study and satellite groups fed the same dietary concentration prior to sampling.

6. Sacrifice and pathology

a. Gross pathology: Pelvic dilatation of the kidney(s) was noted in the treated main study (1/20 treated vs 0/20 controls) and satellite (1/44 treated vs 0/8 controls) groups. No other

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macroscopic findings could be attributed to treatment.

b. Liver enzymes: Tyrosine aminotransferase (TAT) activity was increased in the treated main study and satellite groups (1.316-2.256 mmol HPPA/min/mg protein) compared to controls (1.078-1.991 mmol HPPA/min/mg protein; Table 6a). The activity of 4-hydroxyphenylpyruvate dioxygenase (HPPD) was decreased in the treated main study and satellite groups (0.017-0.218) compared to controls (0.912-1.205); this inhibition appeared to be dose-dependent (Table 6b).

TABLE 6a. Mean (± SD) activities of the liver enzymes, tyrosine aminotransferase and 4-hydroxyphenylpyruvate dioxygenase, in male rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Group		Study day	Last dose mesotrione prior to sampling (ppm)	Mean ± SD
Tyrosine Aminotransferase (mmol HPPA/min/mg protein)				
Control -	Main study	29	0	1.991 ± 0.371
	Satellite study	3	0	1.078 ± 0.188
		15	0	1.323 ± 0.162
Treated -	Main study	29	0.3	2.001 ± 0.572
	Satellite study	3	100	1.893 ± 0.131
		5	30	2.256 ± 0.204
		7	10	2.224 ± 0.195
		9	3	1.860 ± 0.420
		11	1	1.763 ± 0.378
		15	0.3	1.551 ± 0.262
		17	100	2.174 ± 0.314
		19	30	2.252 ± 0.127
		21	10	1.675 ± 0.102
		23	3	1.594 ± 0.162
		25	1	1.316 ± 0.058
4-Hydroxyphenylpyruvate Dioxygenase (µL O_2/min/mg protein)				
Control -	Main study	29	0	0.912 ± 0.167
	Satellite study	3	0	1.190 ± 0.065
		15	0	1.205 ± 0.083
Treated -	Main study	29	0.3	0.131 ± 0.042
	Satellite study	3	100	0.024 ± 0.006
		5	30	0.037 ± 0.013
		7	10	0.055 ± 0.012
		9	3	0.090 ± 0.035
		11	1	0.080 ± 0.035
		15	0.3	0.218 ± 0.029
		17	100	0.017 ± 0.004
		19	30	0.041 ± 0.009
		21	10	0.058 ± 0.005
		23	3	0.066 ± 0.015
		25	1	0.070 ± 0.014

a Data were obtained from Tables 6 and 7 on pages 38-39 in the study report; n = 20 for main study groups, n = 4 for satellite groups.

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TABLE 6b. Mean (± SD) activities of the liver enzymes, tyrosine aminotransferase and 4-hydroxyphenylpyruvate dioxygenase, in male rats fed a variable concentration of mesotrione in the diet for up to 28 days [a]

Nominal concentration in diet (ppm) [b]	Liver enzyme activity
Tyrosine Aminotransferase (mmol HPPA/min/mg protein)	
0	1.46 ± 0.47
0.3	1.78 ± 0.32
1	1.54 ± 0.32
3	1.73 ± 0.19
10	1.95 ± 0.39
30	2.25 ± 0.00
100	2.03 ± 0.20
4-Hydroxyphenylpyruvate Dioxygenase (µL O_2/min/mg protein)	
0	0.60 ± 0.17
0.3	0.17 ± 0.06
1	0.08 ± 0.01
3	0.08 ± 0.02
10	0.06 ± 0.00
30	0.04 ± 0.00
100	0.02 ± 0.00

a Data were calculated by the reviewers from data presented in Table 6a of this DER by averaging the means from rats fed the same dietary concentration prior to sampling.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Administration of variable concentrations of mesotrione in the diet (0.3-100 ppm) for 28 days was generally well tolerated, resulting in slight decreases in body weight gains and food consumption. There were no treatment-related ocular or gross pathological lesions. Plasma tyrosine levels, indicative of tyrosinaemia, corresponded to dietary levels of mesotrione. This effect was due to treatment-related inhibition of liver HPPD, an enzyme involved in the catabolism of tyrosine. A consequence of this inhibition, the induction of liver TAT, was also observed.

B. REVIEWER COMMENTS: No treatment-related effects were observed in the eyes of treated animals in the main study.

Body weights were decreased (↓1-4%; $p \leq 0.05$) in the treated main study animals, resulting in decreased (↓8%) overall body weight gains. Food consumption was decreased (↓4-6%; $p \leq 0.05$) in the treated main study animals on Days 1, 2, and 10. The overall (Days 1-28) mean achieved dose for the treated animals was 2.387 mg/kg/day. Pelvic dilatation of the kidney(s) was noted in the treated main study (1/20 treated vs 0/20 controls) and satellite (1/44 treated vs 0/8 controls) groups.

The activity of HPPD was decreased in the treated main study and satellite groups (0.017-0.218)

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compared to controls (0.912-1.205); this inhibition appeared to be dose-dependent. HPPD is involved in tyrosine catabolism. Thus, HPPD inhibition resulted in plasma tyrosine levels that were increased dose-dependently with increasing concentration of mesotrione in the diet. A consequence of increased plasma concentrations of tyrosine was the increase in activity of TAT in the treated main study and satellite groups (1.316-2.256 mmol HPPA/min/mg protein) compared to controls (1.078-1.991 mmol HPPA/min/mg protein).

This study is classified as an **acceptable/non-guideline** study in the rat.

C. STUDY DEFICIENCIES: The following deficiency was noted, but does not alter the conclusions of this DER:

- Concentration analyses were not performed on test diet samples taken during the study. However, the purpose of this study was to investigate the tyrosinaemia in rats exposed to the test substance and the involvement of liver enzymes in this process. Since the dosing was variable and since setting a LOAEL was not one of the purposes of this study, the lack of concentration analyses on the test diets does not affect the study's acceptability.

APPENDIX

Table 1A. Study design and schedule [a]

Study Day	# Treated Males		Nominal Conc. (ppm)	Dose received (mg/kg/day)	# Control Males		Nominal Conc. (ppm)
	Main Study	Satellite			Main Study	Satellite	
1	20	44	100	13.029	20	8	0
2	20	44	100	12.593	20	8	0
3 [b, c]	20	40	30	3.822	20	4	0
4	20	40	30	3.683	20	4	0
5 [b]	20	36	10	1.273	20	4	0
6	20	36	10	1.208	20	4	0
7 [b]	20	32	3	0.364	20	4	0
8	20	32	3	0.371	20	4	0
9 [b]	20	28	1	0.121	20	4	0
10	20	28	1	0.118	20	4	0
11 [b]	20	24	0.3	0.036	20	4	0
12	20	24	0.3	0.034	20	4	0
13	20	24	0.3	0.034	20	4	0
14	20	24	0.3	0.033	20	4	0
15 [b, c]	20	20	100	10.636	20	0	0
16	20	20	100	10.414	20	0	0
17 [b]	20	16	30	3.097	20	0	0
18	20	16	30	3.140	20	0	0
19 [b]	20	12	10	1.014	20	0	0
20	20	12	10	0.954	20	0	0
21 [b]	20	8	3	0.282	20	0	0
22	20	8	3	0.278	20	0	0
23 [b]	20	4	1	0.094	20	0	0
24	20	4	1	0.090	20	0	0
25 [b]	20	0	0.3	0.028	20	0	0
26	20	0	0.3	0.026	20	0	0
27	20	0	0.3	0.026	20	0	0
28	20	0	0.3	0.025	20	0	0

a Data obtained from Study Report, pages 15, 16, 18, and 59. All main study animals were sacrificed on Day 29

b On these days, 4 rats/group were sacrificed in the treated main study and satellite groups prior to changing the dietary dose level.

c On these days, 4 rats/group were sacrificed in the control main study and satellite groups.

PC 122990

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DATA EVALUATION RECORD

ZA1296 (MESOTRIONE)

Study Type: Non-guideline Reproduction Study in Rats

Work Assignment No. 1-01-16 B (MRID 45651805)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 1, Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050845

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects in the Rat; Non-guideline

PC CODE: 122990 **DP BARCODE:** D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): ZA1296 technical (Mesotrione; 96.8% a.i.)

SYNONYMS: 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Williams, J. (2000) ZA1296: reproductive study in the pregnant rat in conjuction with tyrosine. Central toxicology laboratory, Cheshire, UK. Laboratory Study Id.: CTL Study Number 1356, Syngenta Number 857-97, September 27, 2000. MRID 45651805. Unpublished

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC

EXECUTIVE SUMMARY: The purpose of this study was to evaluate the role of tyrosine in the ZA1296 induced reproductive effects by adding different dose levels of tyrosine in conjunction with ZA1296 in order to exacerbate any possible tyrosine related effects including decreased litter size, decreased pup survival, and bilateral hydronephrosis in the kidneys.

Treatment-related effects of the test substance, tyrosine, and the interaction of the test substance with different levels of tyrosine were examined in a 2 x 4 factorial study design. P females were fed one of 8 possible experimental diets containing the test substance (0 or 2500 ppm) and tyrosine (0, 0.5, 1.0, or 2.0%) from the day of arrival at the performing laboratory on gestation day (GD) 1 until study termination on lactation day (LD) 29. Maternal clinical observations, food consumption, and body weights were measured throughout the study. The dams were allowed to litter naturally; clinical observations, litter size, pup survival at post-natal day (PND) 22, and body weights were measured in the offspring. Plasma tyrosine levels were measured in the P dams on GD 3 and LD 29 (Study Day 51) and in the offspring on PND 29. Pelvic dilatation was examined in the offspring.

MATERNAL ANIMALS: One animal in the 0 ppm ZA1296/2% tyrosine group was found dead on GD 25, and one animal in the 2500 ppm ZA 1296/0% tyrosine group was terminated on GD 23 due to difficulties in parturition. All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 following rapid deterioration (i.e., hunched posture, piloerection, and severe eye lesions) during the first week of the study.

Dietary exposure to ZA1296, in the presence or absence of tyrosine, resulted in opacity in the eyes. Hunched posture was noted in 1/20 rat/group for 1-2 days in the groups treated with tyrosine in the absence of ZA1296. This clinical sign was also noted in the group exposed to ZA1296 alone (1/20 rats for 1 day) and tended to increase in incidence and frequency with increasing tyrosine concentration in the ZA1296 treated animals (1-5/20 rats for 1-12 days vs 0 controls).

Maternal body weights were decreased by 2-8% in the groups exposed to ZA1296 in the presence or absence of tyrosine during GD 4, 7, and 15 and in dams exposed to both ZA1296 and tyrosine generally throughout lactation. Body weight gain for the overall (LD 1-29) lactation period in the 2500 ppm ZA1296/1% tyrosine group was decreased by 54% compared to controls. Maternal food consumption was decreased by 11-14% in the dams fed 2500 ppm ZA1296 in the absence of tyrosine during Week 1 of gestation and Week 3 of lactation. The effect of ZA1296 on food consumption was more frequent and more pronounced with increasing concentration of tyrosine (0.5-1% tyrosine), with decreases of 13-23% during Weeks 1 and 2 of gestation and decreases of 17-53% throughout lactation.

In the absence of ZA1296, plasma tyrosine levels were increased over controls on GD 3 in the 2% tyrosine dams (293 nmol/mL treated vs 182 nmol/mL controls) and on LD 29 in the 1% and 2% tyrosine dams (146 nmol/mL each treated vs 109 nmol/mL controls). In the presence of ZA1296, the increases in plasma tyrosine levels were more pronounced, irrespective of tyrosine levels in the diet (2010-3475 nmol/mL treated vs 109-182 nmol/mL controls).

OFFSPRING: Viability at birth and survival during the post-natal period were affected by treatment with ZA1296 and/or tyrosine. The proportion of pups born live was decreased in the 2% tyrosine group and in the ZA1296 groups with 0.5 and 1% tyrosine. Additionally in the 1% tyrosine/2500 ppm ZA1296 group, decreases were observed in the percentage of pups born live (86.2% treated vs 97.8% controls) and in the proportion of litters with all pups born live (6/18 treated vs 16/19 controls). The following offspring survival parameters were decreased ($p<=0.01$): (i) litter size throughout the post-natal period after PND 1 in the ZA1296 groups with 0.5% tyrosine (decr. 40-58%) and 1% tyrosine (decr. 74-84%); (ii) proportion of pups surviving to PND 22 in the ZA1296 treated groups in the presence or absence of tyrosine and in the >=1% tyrosine groups not fed ZA1296; (iii) percentage of pups surviving to PND 22 in the ZA1296 groups with 0.5 and 1% tyrosine (31.6-59.3% treated vs 93.1% controls); and (iv) proportion of litters with 100% survival in the 1% tyrosine/2500 ppm ZA1296 group (0/18 treated vs 12/19 controls). The decreases in offspring survival were reflected in decreased litter weights in the ZA1296 group with 0.5% tyrosine generally after PND 1 (decr. 20-36%) and in the ZA1296 group with 1% tyrosine throughout the post-natal period (decr. 18-66%); these decreases became

more severe with time. Pup body weights were comparable to controls in both sexes throughout the post-natal period.

Clinical signs of toxicity were limited to offspring from dams fed ZA1296. Cloudy and/or opaque eyes were noted in offspring exposed to ZA1296 in the presence or absence of tyrosine (2-10 pups; 9-70 observations) vs 0 controls. Shaking and piloerection were observed in the 1% tyrosine/2500 ppm ZA1296 group (2 pups; 3 observations).

In offspring not exposed to ZA1296, plasma tyrosine levels were increased in the males at all tyrosine concentrations and in the females at 1 and 2% tyrosine (163-227 nmol/mL treated vs 127-128 nmol/mL controls). Tyrosine levels were increased much more severely in offspring exposed to ZA1296, irrespective of tyrosine concentration (1942-2169 nmol/mL treated vs 127-128 nmol/mL controls).

Incidences of pelvic dilatation in the kidneys were observed in the male and female pups treated with ZA1296 in the presence or absence of tyrosine (33-52% vs 0-10% controls). There were no treatment-related effects of tyrosine alone on pelvic dilatation in the kidneys in either sex but doses of tyrosine alone were much lower than when combined with ZA1296.

In conclusion, this study was successful in demonstrating the effects of ZA1296, tyrosine, and the combination of ZA1296 and tyrosine on the parameters observed in the previous reproductive toxicity study including decreased pup survival and litter size and increased incidences of pelvic dilation of the kidneys.

The statistical analyses conducted were not the most appropriate for the study design. This study employed a 2 x 4 factorial design, in which the effects of two levels of ZA1296 and 4 levels of tyrosine were tested in all possible combinations for a total of eight treatments. The Sponsor compared all of the groups using one-way ANOVA or ANCOVA, followed by pair-wise comparisons of each treated group with the control. A more powerful and appropriate statistical test would have been a two-factor test, testing the effects of ZA1296, tyrosine, and the interaction of ZA1296 and tyrosine (ZA1296*tyrosine). However, this deficiency does not affect the conclusions of this DER or the acceptability of the study.

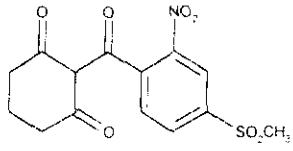
This study is classified as an **acceptable/non-guideline** short-term reproduction study in the rat.

COMPLIANCE - Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: ZA1296 (Mesotrione)

Description:	Light beige powder
Lot/Batch #:	P17
Purity:	96.8% a.i.
Compound Stability:	The test substance was stable in the diet for up to 16 days at -20°C.
CAS #:	104206-82-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat (time-mated females)
Strain:	Alpk:AP$_f$SD
Age at study initiation:	10-12 weeks
Wt. at study initiation:	220-300 g
Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
Housing:	Individually prior to parturition; with litter after parturition. From GD 15, dams were provided with solid cage floors and bedding material.
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22±3°C **Humidity:** 30-70% **Air changes:** ≥15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	None; rats were started on study upon arrival at Central Toxicology Laboratory

B. PROCEDURES AND STUDY DESIGN

1. Purpose: The purpose of this study was to evaluate the role of tyrosine in the ZA1296 induced reproductive effects by adding different dose levels of tyrosine in conjunction with ZA1296 in order to exacerbate any possible tyrosine related effects including decreased litter size, decreased pup survival, and bilateral hydronephrosis in the kidneys. Different dose levels of tyrosine were added in conjunction with the test substance in order to exacerbate any possible tyrosine-related effects. However, the investigators also stated that the choice of dose of ZA1296 was made to specifically investigate litter size and pup survival only.

2. Mating procedure: Nulliparous, nonpregnant females were paired overnight with males of the same strain until insemination was confirmed (by presence of spermatozoa in a daily vaginal smear). Mated females were delivered to the performing laboratory on the day on which evidence of mating was found, designated as gestation day (GD) 1.

3. Study design and schedule: Treatment-related effects of the test substance, tyrosine, and the interaction of the test substance with different levels of tyrosine were examined in a 2 x 4 factorial study design. P females were fed one of 8 possible experimental diets containing the test substance (0 or 2500 ppm) and tyrosine (0, 0.5, 1.0, or 2.0%) from the day of arrival at the performing laboratory (GD 1) until study termination (LD 29). Maternal clinical observations, food consumption, and body weights were measured throughout the study. The dams were allowed to litter naturally; clinical observations, litter size, pup survival (at PND 22), and body weights were measured in the offspring. Plasma tyrosine levels were measured in the P dams on GD 3 and LD 29 (Study Day 51) and in the offspring on PND 29. Pelvic dilatation was examined in the offspring.

4. Animal assignment: Pregnant females were randomly assigned (blocked by location within the experimental array) to the test groups shown in Table 1. Additionally, any females which had been mated with the same male were distributed across the groups.

TABLE 1. Animal assignment [a]

Test Group	Dietary concentration of ZA 1296 (ppm, w/w)	Dietary concentration of Tyrosine (%)	# Females
1	0	0	20
2	0	0.5	20
3	0	1	20
4	0	2	20
5	2500	0	20
6	2500	0.5	20
7	2500	1	20
8	2500	2	20

a Data were obtained from page 17 of the study report.

5. Dose-selection rationale: It was stated that reduced litter size, decreased pup survival, and bilateral hydronephrosis of the kidneys were observed in a previous multi-generation reproductive toxicity study (Study # CTL/P/5147; Milburn, 1997) and were reproduced in a short-term reproductive toxicity study (CTL Study # XR6071). The dose of 2500 ppm ZA1296 was selected because this was the high dose administered in the previous multi-generation reproductive toxicity study (Study # CTL/P/5147; Milburn, 1997). The dietary levels of tyrosine were based on previous studies conducted by the performing laboratory. No further information was provided.

6. Dosage preparation and analysis: The appropriate weighed amount of ZA1296 (corrected for purity) was mixed with diet to make a 2 kg pre-mix. This pre-mix was mixed with additional diet to make a 60 kg batch of diet. Each 60 kg batch of diet (containing either 0 or 2500 ppm ZA1296) was then split into four 15 kg batches. The appropriate amount of tyrosine was added to 1 kg aliquots taken from these 15 kg batches and mixed thoroughly to make a second pre-mix. These second pre-mixes (up to 3 x 1 kg -depending upon the dietary level of tyrosine) were then

mixed back with the diet remaining from the 15 kg batch. The diets were prepared every week or every two weeks and stored at -20°C. The diets were defrosted at room temperature for one hour before use. At one time point during the study, homogeneity (top, middle, bottom) of tyrosine was analyzed for the 0.5 and 2.0% tyrosine diets. Stability of tyrosine in the diet at 0.5 and 2.0% (in the absence of ZA1296) and at 0.5% (in the presence of 2500 ppm ZA1296) was determined for up to 16 days (storage conditions not specified). It was stated that stability (for up to 40 days at -20°C followed by up to 7 days at room temperature) and homogeneity of ZA1296 were determined in previous studies; no data or references were provided. However, in a previously reviewed combined chronic toxicity/oncogenicity study (MRIDs 44505035 and 44505036), homogeneity (top, middle, bottom) was determined by analyzing samples from the 2500, 2.5, and 1 ppm dose formulations. Stability was determined at concentrations of 7000 and 1 ppm over a period of up to 16 days at room temperature and up to 40 days at -20°C. Concentration analyses for ZA1296 and tyrosine were performed on samples from all test diets prepared the week prior to study initiation.

Results:

Homogeneity (range as mean % of nominal)
ZA1296: 75-106%
Tyrosine: 97.3-100.7% nominal; 1.1-2.4% C.V.

Stability (range as mean % day 0)
ZA1296: 84.8-107.4% at room temperature
87.5-114.6% at -20°C
Tyrosine: 100.0-107.4% after 16 days (storage conditions not provided)

Concentration (% of nominal)
ZA1296: 93.8-98.2%
Tyrosine: 93.0-98.0%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental animals: Each rat was observed daily for mortality and clinical signs of toxicity. Body weight measurements and detailed clinical examinations were conducted on GD 1, 4, 7, 15, and 22, and on lactation day (LD) 1, 5, 8, 11, 15, 22, and 29. Food consumption was recorded continuously throughout the study and calculated as a weekly mean per cage (g/rat/day). Plasma tyrosine levels were measured in blood samples collected on GD 3 from 3 rats/dose via the tail vein.

2. Litter observations: The following litter parameters (X) were examined (Table 2):

TABLE 2. Litter observations [a]

Observation	Time of observation (lactation day)						
	Day 1	Day 5	Day 8	Day 11	Day 15	Day 22	Day 29
Number of live pups	X	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X	X
Detailed clinical observations	X	X	X	X	X	X	X

a Data were obtained from page 19 in the study report.

Additionally, each litter was examined daily for dead or moribund pups.

3. Postmortem observations

1) Parental animals: Any animal sacrificed prior to scheduled termination (because of complete litter loss, prolonged gestation [i.e., not littered by GD 25], or parturition difficulty) was euthanized by carbon dioxide inhalation followed by cervical dislocation and discarded without further examination. Animals surviving until study termination were sacrificed on LD 29 by inhalation of an anesthetic. Terminal blood samples were collected via cardiac puncture from up to 5 rats/sex/dose to measure serum tyrosine levels, and these animals were discarded without further examination.

2) Offspring: Any pup sacrificed prior to scheduled termination was euthanized by carbon dioxide inhalation followed by cervical dislocation and discarded without further examination. Pups surviving until study termination were sacrificed on PND 29 by inhalation of an anesthetic. Terminal blood samples were collected via cardiac puncture from 5 rats/dose to measure serum tyrosine levels. Pelvic dilatation in the kidneys was examined in up to 22 males and 20 females per dose group (one pup/sex/litter as near as possible).

D. DATA ANALYSIS

1. Statistical analyses: The following statistical tests were applied to the data. All tests were two-sided.

Parameter	Statistical test
Maternal body weights during gestation	Analysis of covariance (ANCOVA), with GD 1 body weight as the covariate followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test
Maternal body weights during lactation Pup body weight per litter	Analysis of variance (ANOVA) on LD/PND 1; ANCOVA on subsequent days with LD/PND 1 body weight as the covariate followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test
Maternal food consumption Maternal and pup plasma tyrosine Litter size Litter weight	ANOVA followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test
Proportion of whole litter losses Proportion of live born pups Proportion of pups surviving Proportion of litters with all pups born live Proportion of litters with all pups surviving	Fisher's Exact Test

This study employed a 2 x 4 factorial design, in which the effects of two levels of ZA1296 and 4 levels of tyrosine were tested in all possible combinations for a total of eight treatments. The Sponsor compared all of the groups using one-way ANOVA or ANCOVA, followed by pair-wise comparisons of each treated group with the control. A more powerful and appropriate statistical test would have been a two-factor test, testing the effects of ZA1296, tyrosine, and the interaction of ZA1296 and tyrosine (ZA1296*tyrosine).

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality: One animal in group 4 (0 ppm ZA1296/2% tyrosine) was found dead on GD 25, and one animal in group 5 (2500 ppm ZA 1296/0% tyrosine) was terminated on GD 23 due to difficulties in parturition (Table 3). All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 following rapid deterioration (i.e., hunched posture, piloerection, and severe eye lesions) during the first week of the study. There were no other treatment-related mortalities.

2. Clinical signs: Dietary exposure to ZA1296, in the presence or absence of tyrosine, resulted in opacity in the eyes (Table 3). Hunched posture was noted in 1/20 rats/group for 1-2 days in the groups treated with tyrosine in the absence of ZA1296. This clinical sign was also noted in the group exposed to ZA1296 alone (1/20 rats for 1 day) and tended to increase in incidence and frequency with increasing tyrosine concentration in the ZA1296 treated animals (1-5/20 rats for 1-12 days vs 0 controls). There were no other treatment-related clinical signs.

TABLE 3. Clinical signs of toxicity in the dams [#affected (#observations)] [a]

Clinical sign	Dose Group							
	Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)			
	0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500	2/2500 [b]
Eye opacity	0 (0)	0 (0)	0 (0)	0 (0)	12 (35)	19 (134)	19 (208)	8 (15)
Hunched posture	0 (0)	1 (2)	1 (1)	1 (1)	1 (1)	1 (8)	2 (12)	5 (10)
Found dead	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Sacrificed early [c]	1 (1)	2 (2)	8 (8)	3 (3)	1 (1)	7 (7)	11 (11)	20 (20)

a Data were obtained from Table 5 on pages 41-43 in the study report; n = 20.

b All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 following rapid deterioration (i.e., hunched posture, piloerection, and severe eye lesions) during the first week of the study.

c All surviving animals which had not produced litters by GD 25 and those which experienced complete litter loss were sacrificed early.

3. <u>Body weight and food consumption</u>: Maternal body weights were decreased ($p \leq 0.05$) in the groups exposed to ZA1296 in the presence or absence of tyrosine during GD 4, 7, and 15 (↓2-7%) and in dams exposed to both ZA1296 and tyrosine generally throughout lactation (↓4-8%; Table 4). Although body weight gains for the overall (GD 1-22) gestation period were comparable to controls, body weight gain for the overall (LD 1-29) lactation period in the 2500 ppm ZA1296/1% tyrosine group was decreased by 54% compared to controls.

Maternal food consumption was decreased (↓11-14%; $p \leq 0.05$) in the dams fed 2500 ppm ZA1296 in the absence of tyrosine during Week 1 of gestation and Week 3 of lactation (Table 5). The effect of ZA1296 on food consumption was more common and more pronounced with increasing concentration of tyrosine (Groups 6 and 7), with decreases of 13-23% during Weeks 1 and 2 of gestation and decreases of 17-53% throughout lactation.

TABLE 4. Maternal body weights and body weight gain (g) during gestation and lactation [a]

Day		Dose Group						
		Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
		0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Gestation								
1	Mean [b]	249.9	247.6	259.3	244.3	257.4	251.6	252.7
	SD	19.6	15.9	19.7	13.3	17.8	16.5	15.1
4	Mean	268.5	266.3	264.6	265.1	262.5*(-2)	254.3**(-5)	254.4**(-5)
7	Mean	280.8	280.4	276.9	281.4	272.6*(-3)	261.5**(-7)	263.2**(-6)
15	Mean	322.6	322.5	316.8	325.4	313.2*(-3)	300.8*(-7)	303.4**(-6)
22	Mean	391.9	389.6	380.3	394.9	389.1	379.2*(-3)	383.2
Gain (GD 1-22)	Mean [c]	140.4	137.5	127.9	143.3	137.0	127.9	131.4
Lactation								
1	Mean [b]	295.2	288.2	302.9	291.3	295.2	298.1	291.5
	SD	20.0	25.4	26.9	27.4	33.9	20.2	29.9
5	Mean	310.5	312.3	311.3	309.0	317.9	298.2*(-4)	293.6*(-5)
8	Mean	322.9	324.5	323.9	324.0	328.7	306.0*(-5)	302.3*(-6)
11	Mean	334.7	337.5	338.7	336.6	340.3	314.0**(-6)	308.1**(-8)
15	Mean	347.0	351.7	351.5	349.2	349.8	326.4**(-6)	320.7**(-8)
22	Mean	349.9	356.6	351.1	350.7	356.7	331.4**(-5)	325.8**(-7)
29	Mean	334.9	338.0	337.1	335.7	344.6	329.9	313.3**(-6)
Gain (LD 1-29)	Mean [c]	40.4	46.4	36.0	42.6	50.9	35.4	18.5 (-54)

a Data were obtained from Tables 6 and 7 on pages 45-46 of the study report. Percent differences from the controls, calculated by the reviewers, are included in parentheses. All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

b After GD 1 (or LD 1), means were adjusted based on GD 1 (or LD 1) body weight.

c Calculated by the reviewers as the difference in unadjusted group mean body weights

TABLE 5. Maternal food consumption (g/rat/day) during gestation and lactation [a]

Week		Dose Group						
		Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
		0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Gestation								
1	Mean	23.7	24.0	23.5	23.7	21.0** (-11)	18.4** (-22)	18.2** (-23)
	SD	3.2	1.8	2.1	2.1	2.3	4.7	1.9
2	Mean	30.1	30.5	29.0	29.8	28.9	26.2** (-13)	26.3** (-13)
	SD	2.7	2.0	2.2	2.6	3.4	2.9	2.2
3	Mean	30.0	29.8	29.0	29.5	29.9	28.4	28.7
	SD	3.1	2.2	2.2	3.2	3.9	2.1	3.0
Lactation								
1	Mean	35.7	35.3	35.5	36.1	35.0	29.8* (-17)	26.1** (-27)
	SD	5.6	4.1	7.5	7.0	4.9	3.9	3.8
2	Mean	58.7	61.4	57.7	61.9	53.7	44.1** (-25)	37.0** (-37)
	SD	9.8	7.5	12.5	11.8	7.4	7.0	7.6
3	Mean	79.4	84.7	77.6	80.8	68.4** (-14)	58.7** (-26)	45.7** (-42)
	SD	14.7	10.5	13.7	12.8	11.0	10.7	9.2
4	Mean	129.9	132.8	130.3	136.6	121.1	95.4** (-27)	61.0** (-53)
	SD	30.5	30.9	27.5	32.6	31.5	24.5	15.8

a Data were obtained from Tables 8 and 9 on pages 47-48 of the study report. Percent differences from the controls, calculated by the reviewers, are included in parentheses. All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

4. Test substance intake: Achieved intake values of ZA1296 or tyrosine were not reported.

5. Plasma tyrosine levels: In the absence of ZA1296, plasma tyrosine levels were increased ($p \leq 0.05$) over controls on GD 3 in the 2% tyrosine dams (293 nmol/mL treated vs 182 nmol/mL controls) and on LD 29 in the 1% and 2% tyrosine dams (146 nmol/mL each treated vs 109 nmol/mL controls; Table 6). In the presence of ZA1296, the increases ($p \leq 0.01$) in plasma tyrosine levels were more pronounced, irrespective of tyrosine levels in the diet (2010-3475 nmol/mL treated vs 109-182 nmol/mL controls), although the highest plasma tyrosine level (3475 nmol/mL) was observed in the group fed ZA1296 and 2% tyrosine.

TABLE 6. Maternal plasma tyrosine levels (nmol/mL) [a]

Study Day		Dose Group							
		Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)			
		0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500	2/2500
GD 3	Mean	182	200	209	293**	2051**	2644**	2010**	3475**
	SD	7	31	14	88	446	305	375	342
	N	3	3	3	3	3	2	2	3
LD 29	Mean	109	124	146*	146*	2029**	2578**	2470**	NA
	SD	11	9	28	41	372	230	284	
	N	5	5	5	5	5	5	5	

a Data were obtained from Table 10 on page 49 of the study report.
NA Not available because all of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11.

B. OFFSPRING

1. Viability and clinical signs: The proportion of pups born live was decreased ($p \leq 0.05$) in the 2% tyrosine group and in the ZA1296 groups with 0.5 and 1% tyrosine (Table 7). Additionally in the 1% tyrosine/2500 ppm ZA1296 group, decreases ($p \leq 0.01$) were observed in the percentage of pups born live (86.2% treated vs 97.8% controls) and in the proportion of litters with all pups born live (6/18 treated vs 16/19 controls). The following offspring survival parameters were decreased ($p \leq 0.01$): (i) litter size throughout the post-natal period after PND 1 in the ZA1296 groups with 0.5% tyrosine (↓40-58%) and 1% tyrosine (↓74-84%); (ii) proportion of pups surviving to PND 22 in the ZA1296 treated groups in the presence or absence of tyrosine and in the ≥1% tyrosine groups not fed ZA1296; (iii) percentage of pups surviving to PND 22 in the ZA1296 groups with 0.5 and 1% tyrosine (31.6-59.3% treated vs 93.1% controls); and (iv) proportion of litters with 100% survival in the 1% tyrosine/2500 ppm ZA1296 group (0/18 treated vs 12/19 controls).

Clinical signs of toxicity were limited to offspring from dams fed ZA1296 with or without tyrosine (Table 8). Cloudy and/or opaque eyes were noted in offspring exposed to ZA1296 in the presence or absence of tyrosine (2-10 pups; 9-70 observations) vs 0 controls. Shaking and piloerection were observed in the 1% tyrosine/2500 ppm ZA1296 group (2 pups; 3 observations). Other than observations related to viability, there were no other clinical signs that could be attributed to treatment with ZA1296 or tyrosine.

TABLE 7. Litter parameters [a]

Parameter	Dose Group						
	Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
	0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Pups born live							
proportion	224/229	216/227	156/163	205/223**	203/216	190/206*	178/203**
percentage (mean±SD)	97.8 ± 5.6	96.2 ± 11.8	96.3 ± 11.5	92.5 ± 24.1	93.3 ± 10.0	92.2 ± 12.4	86.2** ± 20.4
Proportion of litters with all pups born live	16/19	16/19	12/14	15/18	10/18	10/17	6/18**
Proportion of whole litter loss	0/19	1/19	2/14	2/18	1/18	6/17*	10/18**
Litter size (mean ± SD)							
Day 1	11.8 ± 3.2	11.4 ± 3.1	11.1 ± 3.5	11.4 ± 4.6	11.3 ± 3.1	11.2 ± 2.7	9.9 ± 3.9
Day 5	11.1 ± 3.6	10.1 ± 3.7	9.2 ± 5.1	10.2 ± 4.3	9.7 ± 3.4	6.5** ± 4.7 (141)	2.9** ± 3.3 (174)
Day 8	10.9 ± 3.5	10.1 ± 3.7	9.1 ± 5.0	10.1 ± 4.2	9.6 ± 3.4	6.5** ± 4.6 (140)	2.8** ± 3.3 (174)
Day 11	10.9 ± 3.5	10.1 ± 3.7	9.1 ± 5.0	10.1 ± 4.2	9.6 ± 3.4	6.5** ± 4.6 (140)	2.8** ± 3.3 (174)
Day 15	10.9 ± 3.5	10.1 ± 3.7	9.1 ± 5.0	10.1 ± 4.2	9.6 ± 3.4	6.5** ± 4.6 (140)	2.8** ± 3.3 (174)
Day 22	10.9 ± 3.5	10.1 ± 3.7	9.1 ± 5.0	10.1 ± 4.2	8.9 ± 4.0	5.6** ± 4.5 (149)	2.3** ± 3.0 (179)
Day 29	10.9 ± 3.5	10.1 ± 3.7	9.1 ± 5.0	10.0 ± 4.2	8.8 ± 4.0	4.6** ± 4.2 (158)	1.7** ± 2.3 (184)
Pups surviving to PND 22							
proportion	211/224	192/216	129/156**	174/205**	174/203**	111/190**	52/178**
percentage (mean±SD)	93.1 ± 12.7	87.8 ± 22.1	83.2 ± 36.0	85.9 ± 24.6	85.5 ± 18.2	59.3** ± 39.7	31.6** ± 34.3
Proportion of litters with all pups surviving	12/19	6/19	10/14	9/17	6/18	4/17	0/18**

a Data were obtained from Tables 12 through 15 on pages 56, 57, 59, and 61 in the study report. All of the dams in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

* Significantly different from the controls at p< 0.05

** Significantly different from the controls at p< 0.01

TABLE 8. Clinical signs of toxicity in the offspring [#affected (#observations)] [a]

Clinical sign	Dose Group						
	Tyrosine (%)/ZA1296 ppm				Tyrosine (%)/ZA1296 ppm		
	0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Eye - cloudiness	0 (0)	0 (0)	0 (0)	0 (0)	7 (51)	3 (19)	2 (9)
opacity	0 (0)	0 (0)	0 (0)	0 (0)	8 (70)	10 (70)	8 (31)
Shaking	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3)
Piloerection	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3)

a Data were obtained from Table 11 on pages 51-55 in the study report. All of the dams in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

2. Body weights: Litter weights were decreased ($p \leq 0.05$) in the ZA1296 group with 0.5% tyrosine generally after PND 1 (↓20-36%) and in the ZA1296 group with 1% tyrosine throughout the post-natal period (↓18-66%); these decreases became more severe with time (Table 9). However, because pup weights were comparable to controls throughout the post-natal period for both sexes, these decreases reflect the fact that there were fewer pups in these groups, due to decreased survival, and not actual decreases in pup body weights.

TABLE 9. Mean (±SD) litter weights (g) [a]

PND		Dose Group						
		Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
		0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
1	MEAN	67.9	66.0	64.0	68.5	66.1	65.9	55.4* (↓18)
	S.D.	17.7	16.6	18.2	21.1	16.8	15.8	21.8
	N	19	19	14	17	18	17	18
5	MEAN	92.2	92.2	92.2	93.2	84.7	73.5* (↓20)	43.3** (↓53)
	S.D.	28.1	20.7	28.2	30.0	29.7	25.8	22.6
	N	19	18	12	16	18	13	10
8	MEAN	127.1	128.1	127.1	132.1	118.8	103.6	67.3** (↓47)
	S.D.	35.8	27.2	39.4	40.3	38.7	36.2	26.8
	N	19	18	12	16	18	13	9
11	MEAN	174.6	176.3	173.1	174.2	159.9	138.9* (↓20)	91.3** (↓48)
	S.D.	46.5	38.6	48.7	55.9	51.0	45.1	35.4
	N	19	18	12	16	18	13	9
15	MEAN	246.6	254.0	241.2	251.6	220.6	189.2** (↓23)	129.7** (↓47)
	S.D.	62.1	48.4	61.6	68.7	61.7	54.6	47.2
	N	19	18	12	16	18	13	9
22	MEAN	375.3	400.8	370.9	395.3	320.6	268.6** (↓28)	183.5** (↓51)
	S.D.	93.2	74.1	83.4	104.2	83.7	67.8	61.1
	N	19	18	12	16	17	12	8
29	MEAN	711.5	752.1	710.1	749.6	606.4	457.0** (↓36)	241.5** (↓66)
	S.D.	187.6	152.6	168.5	205.5	171.0	154.9	122.3
	N	19	18	12	16	17	11	8

a Data were obtained from Table 17 on page 64 in the study report. Percent difference from the controls, calculated by the reviewers, is included in parentheses. All of the dams in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

* Statistically different from control, $p \leq 0.05$

** Statistically different from control, $p \leq 0.01$

3. Plasma tyrosine levels: In offspring not exposed to ZA1296, plasma tyrosine levels were increased in the males at all tyrosine concentrations and in the females at 1 and 2% tyrosine (163-227 nmol/mL treated vs 127-128 nmol/mL controls; Table 10). Tyrosine levels were increased much more severely in offspring exposed to ZA1296, irrespective of tyrosine concentration (1942-2169 nmol/mL treated vs 127-128 nmol/mL controls).

Table 10. Offspring plasma tyrosine levels (nmol/mL) on PND 29 [a]

Gender		Dose group						
		Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
		0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Males	Mean	128	163*	224**	210**	2169**	2150**	2114**
	SD	13	16	92	47	116	112	65
	N	5	5	5	5	5	6	6
Females	Mean	127	163	222**	227**	1998**	2061**	1942**
	SD	14	16	103	61	127	145	216
	N	5	5	5	6	5	6	5

a Data were obtained from table 18 on page 65 of the study report. All of the dams in the 2500 ppm za1296/2% tyrosine group were terminated by gd 11 and are thus not included in the table.

4. Pelvic dilatation: Incidences of pelvic dilatation in the kidneys were observed in the male and female pups treated with ZA1296 in the presence or absence of tyrosine (33-52% vs 0-10% controls; Table 11). There were no treatment-related effects of tyrosine alone on pelvic dilatation in the kidneys in either sex.

TABLE 11. Incidences of bilateral pelvic dilatation in the kidneys of the offspring [#affected/#examined (%)] [a]

Gender	Dose Group						
	Tyrosine (%)/ZA1296 (ppm)				Tyrosine (%)/ZA1296 (ppm)		
	0/0	0.5/0	1/0	2/0	0/2500	0.5/2500	1/2500
Males	0/20 (0)	0/20 (0)	0/20 (0)	1/20 (5)	8/22 (36)	9/20 (45)	11/21 (52)
Females	2/20 (10)	1/20 (5)	0/20 (0)	2/20 (10)	7/19 (37)	9/20 (45)	6/18 (33)
Total	2/40 (5)	1/40 (3)	0/40 (0)	3/40 (8)	15/41 (37)	18/40 (45)	17/39 (44)

a Data were obtained from Table 19 on page 67 of the study report. All of the dams in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 and are thus not included in the table.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: This study provided evidence of a causal relationship between tyrosine and the reduced litter size and increased perinatal mortality observed in a previously conducted multi-generation reproduction toxicity study in the rat (Study # CTL/P/5147; Milburn, 1997). A direct causal relationship between tyrosine and bilateral hydronephrosis was less convincingly demonstrated in this study although the choice of dose of ZA1296 was made to specifically investigate litter size and pup survival.

B. REVIEWER COMMENTS

1. PARENTAL ANIMALS: One animal in the 0 ppm ZA1296/2% tyrosine group was found dead on GD 25, and one animal in the 2500 ppm ZA 1296/0% tyrosine group was terminated on

GD 23 due to difficulties in parturition. All of the rats in the 2500 ppm ZA1296/2% tyrosine group were terminated by GD 11 following rapid deterioration (i.e., hunched posture, piloerection, and severe eye lesions) during the first week of the study.

Dietary exposure to ZA1296, in the presence or absence of tyrosine, resulted in opacity in the eyes. Hunched posture was noted in 1/20 rats/group for 1-2 days in the groups treated with tyrosine in the absence of ZA1296. This clinical sign was also noted in the group exposed to ZA1296 alone (1/20 rats for 1 day) and tended to increase in incidence and frequency with increasing tyrosine concentration in the ZA1296 treated animals (1-5/20 rats for 1-12 days vs 0 controls).

Maternal body weights were decreased (↓2-8%; $p \leq 0.05$) in the groups exposed to ZA1296 in the presence or absence of tyrosine during GD 4, 7, and 15 and in dams exposed to both ZA1296 and tyrosine generally throughout lactation. Body weight gain for the overall (LD 1-29) lactation period in the 2500 ppm ZA1296/1% tyrosine group was decreased by 54% compared to controls. Maternal food consumption was decreased (↓11-14%; $p \leq 0.05$) in the dams fed 2500 ppm ZA1296 in the absence of tyrosine during Week 1 of gestation and Week 3 of lactation. The effect of ZA1296 on food consumption was more frequent and more pronounced with increasing concentration of tyrosine (0.5-1% tyrosine), with decreases of 13-23% during Weeks 1 and 2 of gestation and decreases of 17-53% throughout lactation.

In the absence of ZA1296, plasma tyrosine levels were increased ($p \leq 0.05$) over controls on GD 3 in the 2% tyrosine dams (293 nmol/mL treated vs 182 nmol/mL controls) and on LD 29 in the 1% and 2% tyrosine dams (146 nmol/mL each treated vs 109 nmol/mL controls). In the presence of ZA1296, the increases ($p \leq 0.01$) in plasma tyrosine levels were more pronounced, irrespective of tyrosine levels in the diet (2010-3475 nmol/mL treated vs 109-182 nmol/mL controls), although the highest plasma tyrosine level (3475 nmol/mL) was observed in the group fed ZA1296 and 2% tyrosine.

2. <u>OFFSPRING</u>: The proportion of pups born live was decreased ($p \leq 0.05$) in the 2% tyrosine group and in the ZA1296 groups with 0.5 and 1% tyrosine. Additionally in the 1% tyrosine/2500 ppm ZA1296 group, decreases ($p \leq 0.01$) were observed in the percentage of pups born live (86.2% treated vs 97.8% controls) and in the proportion of litters with all pups born live (6/18 treated vs 16/19 controls). The following offspring survival parameters were decreased ($p \leq 0.01$): (i) litter size throughout the post-natal period after PND 1 in the ZA1296 groups with 0.5% tyrosine (↓40-58%) and 1% tyrosine (↓74-84%); (ii) proportion of pups surviving to PND 22 in the ZA1296 treated groups in the presence or absence of tyrosine and in the ≥1% tyrosine groups not fed ZA1296; (iii) percentage of pups surviving to PND 22 in the ZA1296 groups with 0.5 and 1% tyrosine (31.6-59.3% treated vs 93.1% controls); and (iv) proportion of litters with 100% survival in the 1% tyrosine/2500 ppm ZA1296 group (0/18 treated vs 12/19 controls). The decreases in offspring survival were reflected in decreased ($p \leq 0.05$) litter weights in the ZA1296 group with 0.5% tyrosine generally after PND 1 (↓20-36%) and in the ZA1296 group with 1% tyrosine throughout the post-natal period (↓18-66%); these decreases became more severe with

time. Pup body weights were comparable to controls in both sexes throughout the post-natal period.

Clinical signs of toxicity were limited to offspring from dams fed ZA1296 (with or without tyrosine). Cloudy and/or opaque eyes were noted in offspring exposed to ZA1296 in the presence or absence of tyrosine (2-10 pups; 9-70 observations) vs 0 controls. Shaking and piloerection were observed in the 1% tyrosine/2500 ppm ZA1296 group (2 pups; 3 observations).

In offspring not exposed to ZA1296, plasma tyrosine levels were increased in the males at all tyrosine concentrations and in the females at 1 and 2% tyrosine (163-227 nmol/mL treated vs 127-128 nmol/mL controls). Tyrosine levels were increased much more severely in offspring exposed to ZA1296, irrespective of tyrosine concentration (1942-2169 nmol/mL treated vs 127-128 nmol/mL controls).

Incidences of pelvic dilatation in the kidneys were observed in the male and female pups treated with ZA1296 in the presence or absence of tyrosine (33-52% vs 0-10% controls). There were no treatment-related effects of tyrosine alone on pelvic dilatation in the kidneys in either sex but doses of tyrosine alone were much lower than when combined with ZA1296.

In summary, this study was successful in demonstrating the effects of ZA1296, tyrosine, and the combination of ZA1296 and tyrosine on the parameters observed in the previous reproductive toxicity study including decreased pup survival and litter size and increased incidences of pelvic dilation of the kidneys.

This study is classified as an **acceptable/non-guideline** short-term reproduction study in the rat.

C. <u>STUDY DEFICIENCIES</u>: The following deficiency was noted but does not alter the conclusions of this DER:

- The statistical analyses conducted were not the most appropriate for the study design. This study employed a 2 x 4 factorial design, in which the effects of two levels of ZA1296 and 4 levels of tyrosine were tested in all possible combinations for a total of eight treatments. The Sponsor compared all of the groups using one-way ANOVA or ANCOVA, followed by pair-wise comparisons of each treated group with the control. A more powerful and appropriate statistical test would have been a two-factor test, testing the effects of ZA1296, tyrosine, and the interaction of ZA1296 and tyrosine (ZA1296*tyrosine).

DATA EVALUATION RECORD

ZA1296 (MESOTRIONE)

Study Type: Non-guideline Study; Investigation of Liver and Kidney Enzyme Parameters in Control Mouse Pups

Work Assignment No. 1-01-16 C (MRID 45651807)

Prepared for
Health Effects Division
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050845

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline Study; Investigation of Liver and Kidney Enzyme Parameters - Mice.

PC CODE: 122990

DP BARCODE: D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): Untreated

SYNONYMS: None

CITATION: Williams, J. (2001) Investigation of liver and kidney enzyme parameters in control mouse pups from new born to age 42 days. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study Id.: CTL Study No. RM0801, Syngenta No. 1251-98, January 23, 2001. MRID 45651807. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC.

EXECUTIVE SUMMARY: In a non-guideline study (MRID 45651807), 54 untreated pregnant Alpk:AP$_f$CD-1 female mice were allowed to litter normally. Six females and their litters were killed on postnatal days (PND) 1, 2, 3, 4, 5, and 8; three females and their litters were killed on PND 12, 15, 22, 29, 35, and 42. These animals were exsanguinated, and the liver and kidneys were removed. The levels of plasma tyrosine, liver and kidney tyrosine aminotransferase (TAT) activity, and liver and kidney 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity were determined. The purpose of this study was to provide a database on the basal levels of plasma tyrosine and liver and kidney enzymes (TAT and HPPD) involved in tyrosine catabolism in control maternal mice and mouse pups up to 42 days of age.

Maternal plasma tyrosine levels appeared variable with standard deviations reaching ± 26.2 on PND 15. They appeared to rise from PND 1 to PND 3, although variations continued to be large expecially as noted on PND 29. Maternal liver TAT activity peaked on PND 2, and on PND 3 began to decrease with some level of stability by PND 29. Maternal kidney TAT activity also was variable. It peaked on PND 3, fell through PND 15, then rose through PND 42. Liver HPPD activity appeared less variable throughout the study. Kidney HPPD activity was much

lower than in the liver and but still appeared variable over time. Kidney TAT activity was approximately 8-fold lower than that observed in liver, while kidney HPPD activity was substantially (approximately 44-fold) lower than in liver.

Pup plasma tyrosine levels rose steadily from birth, peaking on PND 12 for both sexes. However, values were also variable with plasma tyrosine levels then falling through PND 29 and then rising somewhat again near the end of the sampling period on day 42 in both sexes. In general, pup plasma tyrosine levels were higher for PND 1-42 than that observed in maternal females. Liver TAT activity appeared to fluctuate greatly throughout the study, with males and females exhibiting low values on PND 12 and high values on PND 29. In general, liver TAT activity in both sexes was lower than that observed in adult females although there was some overlap of values. Kidney TAT activity was also quite variable. It rose from a low on PND 1-2, approached adult female levels by PND 4, and fluctuated for the remainder of the study. Liver HPPD activity rose from a low on PND 1-2, approached adult female levels by PND 22, and fluctuated widely for the remainder of the study. Kidney HPPD activity rose from a low on PND 1, approached adult female levels by PND 4, and fluctuated widely for the remainder of the study. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities.

In summary, in neonatal pups, plasma tyrosine levels rose steadily from birth until PND 12 and appeared to become lower and more variable by the end of the sampling period. In both sexes, plasma tyrosine levels appeared slightly higher at PND 42 than that observed in maternal females. In general, liver TAT activity in both sexes was lower than that observed in adult females. Kidney TAT activity approached adult female levels by PND 4. Liver HPPD activity approached adult female levels by PND 22, while kidney HPPD activity approached adult female levels by PND 4. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities. In addition, results during many sampling periods varied widely and it is unclear whether this is associated with biochemical analyses methodology problems, small sample size or whether the values actually varied within the test population at the intervals sampled. Replicate analyses would be necessary to confirm results. For the above listed reasons, it is unclear whether useful background data have been obtained in this study.

The submitted study is classified as **acceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Flagging statements were provided. It was stated that this study was not conducted in compliance with any GLP regulations (i.e., US EPA 40 CFR Parts 160 and 792) and was not audited by the QA unit of the performing lab; however, it was conducted to the highest standards of research practices.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: None

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Mouse (female)	
Strain:	Alpk:AP$_f$CD-1	
Age/ mean group weight at arrival:	Age not provided; 50.4 g	
Source:	Rodent Breeding Unit, Alderly Park, Cheshire, UK	
Housing:	Females were individually housed with litters until pups were 29 days old. Pups were then rehoused as litter mates, up to 5/cage.	
Diet:	R&M No. 3 diet (Special Diet Services, Ltd., Witham, Essex, UK), *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	At least 15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	Not applicable	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: 8/12/98 End: 11/3/98

2. Objective: The objective of this study was to provide a database on the basal levels of plasma tyrosine and activities of liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) in control maternal mice and mouse pups up to postnatal day (PND) 42.

3. Mating: Mating was performed by the supplier. A description of mating was not provided; however, the day on which a positive indication of mating (vaginal plug) was observed was designated as gestation day (GD) 1. Animals were delivered to the performing laboratory on GD 14.

4. Study design: Fifty-four pregnant Alpk:AP$_f$CD-1 female mice were allowed to litter normally. Six females and their litters were killed on postnatal days (PND) 1, 2, 3, 4, 5, and 8; three females and their litters were killed on PND 12, 15, 22, 29, 35, and 42. Plasma tyrosine and liver and kidney TAT and HPPD were measured in the dams and the pups.

5. Dosage preparation, administration, and analysis: Not applicable

C. METHODS

1. Maternal evaluations: Mice were checked for mortality and clinical signs of toxicity daily during the study. Detailed observations were recorded when the animals were weighed. Body weights were determined on arrival (GD 14), PND 1, 5, 8, 12, 15, 22, and 29, and at termination. Food consumption was not measured. Six females and their litters were killed on PND 1, 2, 3, 4, 5, and 8; three females and their litters were killed on PND 12, 15, 22, 29, 35, and 42. Females were killed by overexposure to halothane anesthesia and exsanguinated by cardiac puncture, and the liver and kidneys were removed.

2. Pup evaluations: Each litter was examined daily for dead or abnormal pups; these were discarded without examination. Detailed observations were recorded when the pups were weighed. A count of all pups (live and dead) was made within 24 hours of parturition and on PND 5, 8, 12, 15, 22, and 29. Body weights were determined on PND 1, 5, 8, 12, 15, 22, 29, 35, and 42, and at termination. Food consumption after weaning was not measured. For litters terminated on PND 1-8, all pups were killed, and the blood, liver, and kidneys were pooled for each sex/litter. For the 3 litters terminated on PND 12-42, the litter with the most pups was killed first, and blood, liver, and kidneys were taken from each animal. Blood was taken from all pups in the second and third litters, but liver and kidneys were taken from a maximum of 4 pups/sex/litter. Pups at the lower extreme of the weight range in the last 2 litters were not used for tissue collection. Pups were killed by cervical dislocation and exsanguinated by decapitation through PND 8; thereafter, pups were killed by overexposure to halothane anesthesia followed by cardiac puncture.

3. Plasma tyrosine and tissue enzyme activity analysis: Blood samples were pooled for all of the pups of each sex per litter. All blood samples were centrifuged, and the plasma was removed and stored at -20°C until analysis. Plasma tyrosine levels were measured by HPLC with UV detection using a Hichrom S5ODS2 column with a mobile phase gradient of 100% acetonitrile mixing with water:acetonitrile:trifluoroacetic acid (950:50:2; v/v). Livers and kidneys were weighed, homogenized, and centrifuged to yield cytosols that were then aliquoted and stored at -70°C until enzyme analysis was performed. Tyrosine aminotransferase (TAT) catalyzes the conversion of tyrosine to *p*-hydroxyphenylpyruvic acid (HPPA). HPPA will react with phenazine methosulphate to form a colored reaction product that can be measured by a spectrophotometer at 675 nm. Cytosols were analyzed spectrophotometrically (in duplicate) for TAT activity. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) catalyzes the oxidative decarboxylation and rearrangement of HPPA to homogentisate, with incorporation of both atoms of molecular oxygen into the product. Thus, HPPD activity in solution can be measured by monitoring oxygen consumption. Cytosolic oxygen consumption was measured using an oxygen electrode.

D. DATA ANALYSIS: All data were presented as mean values ±SD. As only control animals were evaluated, no statistical analyses were performed.

II. RESULTS

A. MATERNAL EVALUATIONS: No significant clinical signs were observed, and the data were not included in the study report. Individual body weight data were included but not tabulated; however, these data did not contribute to the stated objective of this study.

1. Plasma tyrosine analysis: Maternal plasma tyrosine levels are summarized in Table 1. Plasma tyrosine levels appeared to rise from 48.1 nmol/mL on PND 1 to 62.6 nmol/mL on PND 3.

Table 1. Plasma tyrosine levels (nmol/mL; mean±SD) in maternal mice following parturition.[a]

Day of sacrifice	Tyrosine
PND 1	48.1±11.8
PND 2	59.1±4.8
PND 3	62.6±11.2
PND 4	62.4±8.7
PND 5	62.8±10.0
PND 8	60.6±4.5
PND 12	74.5±18.6
PND 15	71.5±26.2
PND 22	65.2±11.0
PND 29	52.3±5.7
PND 35	64.7±12.8
PND 42	66.4±5.8
Overall[b]	62.5

a Data obtained from page 55 of the study report.
b Calculated by reviewers from data presented in this table

2. TAT activity: Maternal TAT activity in liver and kidney cytosols are summarized in Table 2. Liver TAT activity peaked at 14.01 nmol HPPA/min/mg protein on PND 2 and on PND 3 began to decrease somewhat but values were variable. Kidney TAT levels were approximately 8-fold lower than liver levels. Kidney TAT values were also variable. They peaked on PND 3 (1.309 nmol HPPA/min/mg protein), fell through PND 15 (0.654 nmol HPPA/min/mg protein), then rose again through PND 42 (1.844 nmol HPPA/min/mg protein).

Table 2. TAT activity (nmol HPPA/min/mg protein; mean±SD) in liver and kidney cytosols of maternal mice following parturition.[a]

Day of sacrifice	Liver	Kidney
PND 1	8.291±1.638	0.960±0.285
PND 2	14.014±1.795	1.223±0.431
PND 3	11.918±2.093	1.309±0.333
PND 4	10.828±1.722	1.202±0.184
PND 5	9.721±1.131	0.866±0.378
PND 8	9.461±1.339	0.840±0.129
PND 12	6.181±1.096	0.762±0.172
PND 15	7.050±0.715	0.654±0.144
PND 22	6.557±1.359	0.855±0.158
PND 29	7.401±0.735	0.933±0.061
PND 35	7.636±1.578	1.763±0.313
PND 42	7.654±2.338	1.844±0.649
Overall[b]	8.893	1.101

a Data obtained from pages 72 and 85 of the study report.
b Calculated by reviewers from data presented in this table

3. <u>4-Hydroxyphenylpyruvate dioxygenase (HPPD) activity</u>: Maternal HPPD activity in liver and kidney cytosols are summarized in Table 3. Liver HPPD activity appeared less variable (PND 1-42: 1.369-1.785 µL O_2/min/mg protein). Kidney HPPD activity was substantially (approximately 44-fold) lower than liver levels. Kidney HPPD activity appeared variable although at much lower levels than in the liver (PND 1-12 (0.028-0.041 µL O_2/min/mg protein), fell through PND 15 (0.018 µL O_2/min/mg protein), then rose through PND 35 (0.068 µL O_2/min/mg protein).

Table 3. HPPD activity (µL O_2/min/mg protein; mean±SD) in liver and kidney cytosols of maternal mice following parturition.[a]

Day of sacrifice	Liver	Kidney
PND 1	1.394±0.127	0.032±0.030
PND 2	1.705±0.162	0.029±0.024
PND 3	1.471±0.143	0.028±0.021
PND 4	1.547±0.113	0.041±0.025
PND 5	1.664±0.245	0.028±0.009
PND 8	1.369±0.238	0.033±0.016
PND 12	1.388±0.052	0.033±0.033
PND 15	1.610±0.041	0.018±0.004
PND 22	1.594±0.417	0.019±0.006
PND 29	1.785±0.215	0.040±0.019
PND 35	1.826±0.096	0.068±0.016
PND 42	1.711±0.168	0.065±0.053
Overall[b]	1.589	0.036

a Data obtained from pages 94 and 106 of the study report.
b Calculated by reviewers from data presented in this table

B. PUP EVALUATIONS: It was stated that no significant clinical signs were observed. Individual litter data (body weight, sex, and number of pups) were included but not tabulated; however, these data did not contribute to the stated objective of this study.

1. Plasma tyrosine analysis: Pup plasma tyrosine levels are summarized in Table 4. Plasma tyrosine levels rose steadily from birth, peaking on PND 12 for both sexes (233.6-253.5 nmol/mL). Tyrosine levels then became more variable and fell through PND 29 (48.6-56.5 nmol/mL) In general, pup plasma tyrosine levels were higher for PND 1-42 than that observed in maternal females.

Table 4. Plasma tyrosine levels (nmol/mL; mean±SD) in pups for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	79.7±18.8	75.2±20.6
PND 2	132.2±24.2	118.4±23.4
PND 3	171.7±20.5	144.7±26.0
PND 4	183.0±44.1	172.0±38.6
PND 5	208.7±38.8	198.5±31.1
PND 8	204.3±19.2	203.4±28.2
PND 12	253.5±53.7	233.6±67.3
PND 15	191.8±33.4	162.6±43.0
PND 22	89.9±17.4	87.1±18.7
PND 29	56.5±17.8	48.6±14.4
PND 35	81.9±21.1	81.1±21.7
PND 42	96.2±11.4	81.6±8.9
Overall[b]	145.8	133.9

a Calculated by reviewers from data obtained from pages 65-67 of the study report.
b Calculated by reviewers from data presented in this table

2. <u>Tyrosine aminotransferase (TAT) activity</u>: TAT activity is shown in Tables 5a and b. Liver TAT activity appeared to fluctuate extensively throughout the study, with males and females exhibiting low values on PND 12 (2.051 and 2.268 nmol HPPA/min/mg protein, respectively) and high values on PND 29 (9.632 and 11.082 nmol HPPA/min/mg protein, respectively). In general, liver TAT activity in both sexes was lower than that observed in adult females. Kidney TAT levels were approximately 6-8-fold lower than liver levels. Kidney TAT activity rose from a low on PND 1-2 (0.085-0.264 nmol HPPA/min/mg protein), approached adult female levels by PND 4, and fluctuated for the remainder of the study.

Table 5a. TAT activity (nmol HPPA/min/mg protein: mean±SD) in liver cytosols of pups for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	5.592±2.366	6.863±1.667
PND 2	6.095±3.664	7.078±5.763
PND 3	4.162±0.992	4.104±1.442
PND 4	6.363±3.733	4.581±0.858
PND 5	3.302±1.377	3.724±1.812
PND 8	3.307±0.760	3.879±1.236
PND 12	2.051±0.912	2.268±1.107
PND 15	3.406±1.195	4.017±2.001
PND 22	7.287±2.710	7.321±2.417
PND 29	9.632±4.715	11.082±3.994
PND 35	6.528±1.204	6.067±0.808
PND 42	5.769±1.002	6.095±0.785
Overall[b]	5.291	5.590

a Calculated by reviewers from data obtained from pages 86-88 of the study report.
b Calculated by reviewers from data presented in this table

Table 5b. TAT activity (nmol HPPA/min/mg protein; mean±SD) in kidney cytosols of pups for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	0.218±0.190	0.263±0.133
PND 2	0.085±0.147	0.264±0.141
PND 3	0.677±0.092	0.378±0.151
PND 4	0.635±0.400	1.628±1.421
PND 5	1.015±0.509	0.816±0.108
PND 8	0.605±0.134	0.577±0.262
PND 12	0.601±0.431	0.999±0.521
PND 15	0.885±0.326	1.213±0.575
PND 22	0.992±0.180	0.765±0.304
PND 29	0.729±0.140	0.831±0.254
PND 35	0.804±0.204	1.326±0.432
PND 42	0.592±0.191	1.499±0.538
Overall[b]	0.653	0.880

a Data obtained from page 84 of the study report.
b Calculated by reviewers from data presented in this table

3. <u>4-Hydroxyphenylpyruvate dioxygenase (HPPD) activity</u>: HPPD activity is shown in Tables 6a and b. Liver HPPD activity rose from a low on PND 1-2 (0.462-0.594 µL O_2/min/mg protein), approached adult female levels by PND 22, and fluctuated for the remainder of the study. Kidney HPPD levels were approximately 35-40-fold lower than liver levels. Kidney HPPD activity rose from a low on PND 1 (0.012-0.013 µL O_2/min/mg protein), approached adult female levels by PND 4, and fluctuated for the remainder of the study.

Table 6a. HPPD activity (μL O_2/min/mg protein; mean±SD) in liver cytosols of pups for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	0.565±0.065	0.594±0.053
PND 2	0.462±0.085	0.632±0.183
PND 3	0.744±0.191	0.734±0.194
PND 4	0.862±0.124	0.872±0.172
PND 5	1.067±0.149	1.208±0.263
PND 8	1.012±0.095	1.071±0.111
PND 12	0.881±0.131	0.783±0.137
PND 15	1.156±0.239	1.172±0.226
PND 22	1.972±0.299	2.023±0.294
PND 29	1.711±0.237	1.874±0.168
PND 35	1.494±0.180	1.888±0.228
PND 42	1.280±0.125	1.857±0.184
Overall[b]	1.101	1.226

a Calculated by reviewers from data obtained from pages 107-109 of the study report.
b Calculated by reviewers from data presented in this table

Table 6b. HPPD activity (μL O_2/min/mg protein; mean±SD) in kidney cytosols of pups for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	0.013±0.012	0.012±0.007
PND 2	0.016±0.014	0.017±0.006
PND 3	0.014±0.008	0.012±0.007
PND 4	0.033±0.020	0.047±0.035
PND 5	0.030±0.027	0.033±0.013
PND 8	0.028±0.015	0.012±0.012
PND 12	0.021±0.016	0.016±0.011
PND 15	0.019±0.012	0.014±0.010
PND 22	0.030±0.009	0.032±0.016
PND 29	0.047±0.051	0.071±0.057
PND 35	0.048±0.014	0.093±0.067
PND 42	0.028±0.032	0.066±0.075
Overall[b]	0.027	0.035

a Data obtained from page 105 of the study report.
b Calculated by reviewers from data presented in this table

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The purpose of this study was to provide a database for the basal levels of enzymes (TAT and HPPD) involved in tyrosine catabolism in neonatal and young mice, together with consequential plasma levels of tyrosine. Levels of TAT in neonatal and young mice were comparable to the activity noted in adult mice. Plasma tyrosine levels were inversely related to the activity of this enzyme. Levels of HPPD were lower in mice aged from day 1 to day 15 *post partum* compared with adult mice. For both enzymes, the activities were much higher in the liver than in the kidneys for all mice.

B. REVIEWER COMMENTS:

Maternal plasma tyrosine levels appeared variable with standard deviations reaching ± 26.2 on PND 15. They appeared to rise from PND 1 to PND 3, although variations continued to be large expecially as noted on PND 29. Maternal liver TAT activity peaked on PND 2, and on PND 3 began to decrease with some level of stability by PND 29. Maternal kidney TAT activity also was variable. It peaked on PND 3, fell through PND 15, then rose through PND 42. Liver HPPD activity appeared less variable throughout the study. Kidney HPPD activity was much

lower than in the liver and but still appeared variable over time. Kidney TAT activity was approximately 8-fold lower than that observed in liver, while kidney HPPD activity was substantially (approximately 44-fold) lower than in liver.

Pup plasma tyrosine levels rose steadily from birth, peaking on PND 12 for both sexes. However, values were also variable with plasma tyrosine levels then falling through PND 29 and then rising somewhat again near the end of the sampling period on day 42 in both sexes. In general, pup plasma tyrosine levels were higher for PND 1-42 than that observed in maternal females. Liver TAT activity appeared to fluctuate greatly throughout the study, with males and females exhibiting low values on PND 12 and high values on PND 29. In general, liver TAT activity in both sexes was lower than that observed in adult females although there was some overlap of values. Kidney TAT activity was also quite variable. It rose from a low on PND 1-2, approached adult female levels by PND 4, and fluctuated for the remainder of the study. Liver HPPD activity rose from a low on PND 1-2, approached adult female levels by PND 22, and fluctuated widely for the remainder of the study. Kidney HPPD activity rose from a low on PND 1, approached adult female levels by PND 4, and fluctuated widely for the remainder of the study. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities.

In summary, in neonatal pups, plasma tyrosine levels rose steadily from birth until PND 12 and appeared to become lower and more variable by the end of the sampling period. In both sexes, plasma tyrosine levels appeared slightly higher at PND 42 than that observed in maternal females. In general, liver TAT activity in both sexes was lower than that observed in adult females. Kidney TAT activity approached adult female levels by PND 4. Liver HPPD activity approached adult female levels by PND 22, while kidney HPPD activity approached adult female levels by PND 4. In the kidney, TAT activity was approximately 6-8-fold lower than in liver, while HPPD levels were approximately 35-40-fold lower than in liver. No apparent differences between sexes were observed up to PND 42 in pup plasma tyrosine levels or liver and kidney TAT and HPPD activities. In addition, results during many sampling periods varied widely and it is unclear whether this is associated with biochemical analyses methodology problems, small sample size or whether the values actually varied within the test population at the intervals sampled. Replicate analyses would be necessary to confirm results. For the above listed reasons, it is unclear whether useful background data have been obtained in this study.

The submitted study is classified as **acceptable/non-guideline**.

C. <u>STUDY DEFICIENCIES</u>: This is not a guideline study and it is unclear how useful study data generated might be if used as background data have in this test species.

DATA EVALUATION RECORD

ZA1296 (MESOTRIONE)

Study Type: Non-guideline Study; Method Development of Standard Operating Procedures and Generation of Historical Control Data for Developmental Toxicity Studies in Mice

Work Assignment No. 1-01-16 E and F (MRID 45651810 and 45651808)

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050845

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline Study; Prenatal Developmental Toxicity Study - Mice.

PC CODE: 122990 **DP BARCODE:** D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): Untreated (Water: purity not provided)

SYNONYMS: None

CITATION: Moxon, M. (2001) Prenatal development toxicity: method development study in the mouse to achieve compliance with EPA guideline. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RM0811, Syngenta No. 1254-98, April 10, 2001. MRID 45651810. Unpublished.

Moxon, M..E. (2001) ZA1296: Dose range finding study in mice. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RM0799, Syngenta No. 1252-98, April 10, 2001. MRID 45651808. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC.

EXECUTIVE SUMMARY:

In a non-guideline developmental toxicity study (MRID 45651810), water was administered daily by oral gavage at a dose volume of 1 mL/100 g body weight to 50 Alpk:AP$_f$CD-1 female mice on gestation days (GD) 5 through 18. All mice were sacrificed on GD 19; their fetuses were removed by cesarean and examined. The objective of this study was to establish standard operating procedures for the staining and evaluation of fetal bone and cartilage, and to provide background control data to support future developmental toxicity studies in the mouse.

Ten of the 50 mice did not survive to scheduled termination. One mouse was killed following premature delivery on GD 17, and one mouse died during observation on GD 6. The deaths of the other 8 mice were attributed to poor dosing technique. Two mice were found dead, one each on GD 6 and 13. Six mice were sacrificed due to clinical signs; one on GD 6, two on GD 10, one on GD 11, and two on GD 18. Clinical signs included altered breathing pattern, hunched

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posture, piloerection, subdued behavior, shaking, closed eyes, cold to touch, and pallor. Food consumption was observed to increase slightly over the treatment period. The mice were observed to almost double their body weight by GD 19; however, gravid uterus weight accounted for the majority of the increase. *Post Mortem* macroscopic findings were only reported for 10 of the 50 mice on study (perhaps only those that died on study). This would significantly limit the assessment of potential dosing errors during the study to animals that survived to term. A few findings were noted for mice that survived to term but it does not appear that complete necropsies were performed for 80% of animals on test. One mouse that survived to sacrifice was observed to have a mass in the thoracic cavity that was adherent to the heart, lungs, thoracic wall, and diaphragm, an enlarged spleen, and the liver was adherent to the diaphragm. However, these findings were also considered by the investigators as due to poor dosing technique. In mice that died *in extremis* or were killed prior to study termination, the most common findings, indicative of dosing errors, were adhesions of the lungs, perforated esophagus, excess watery fluid in the thoracic cavity, and adhesions in the thoracic cavity. Since 6 of the 8 deaths attributed to poor dosing technique occurred on or prior to GD 13, the dosing technique was considered by the investigators to have improved to some minimal extent during the latter portion of the study.

The overall pregnancy rate for the study was 74%. The pregnancy rates for mice delivered to the performing laboratory on GD 1, 2, or 3 were 90.5%, 75.0%, and 46.2%, respectively. Due to this substantial decrease in pregnancy rate, it was concluded by the investigators that the optimal time for shipment of pregnant mice was GD 1. However, these differences could have easily been due to numerous other factors including variability in rates at shipment and stress associated with dosing error. In addition, it is clear that 13 of 50 females were not pregnant at term and this left only 37 of 50 animals for litter assessment.

One dam was killed following a premature delivery, and complete resorption was observed in another dam that survived to termination and at necropsy was found to have signs of misdosing. Three fetuses were found to have major external defects (extra digits of the forepaw, malrotated hindlimb, and cleft palate). One fetus was found to have a major visceral defect (missing aortic arch). Six fetuses were found to have major skeletal defects. Four of these fetuses came from one litter of 20 fetuses; all exhibited multiple shortened bones in the fore and hindlimbs, including bilateral curved and shortened radius, bilateral shortened ulna, humerus, femur, fibula, tibia, scapula, and ilium. Fused premaxillae and extra metacarpals and misshapen metacarpals were also noted. The remaining 2 fetuses came from a single litter; one fetus was observed to have thoracic arches 7 and 8 fused and cartilage fused between arches 5 and 6, while the other was noted to have multiple minor defects that were collectively classified as a malformation. Four fetuses were found to have minor external defects (slight flexion of the hindlimb or kink of the tail). Nineteen fetuses were found to have minor visceral defects (discolored thymus or umbilical artery left of the bladder). Numerous skeletal variants and minor defects were observed. The variants occurring with the greatest incidence (>=10% of fetuses) were: (i) centrum 3, 4, and 5 not ossified; (ii) small holes in sternebrae 6; (iii) xiphoid cartilage cleft; (iv) 7th cervical rib shortened; and (v) hindpaw calcaneum ossified. The minor defects occurring with the greatest incidence (>=10% of fetuses) were: (i) small holes in supraoccipital bones; (ii) slight

incomplete ossification of the parietal bones; (iii) sternebrae 4 incompletely cleft; (iv) sternebrae 5 and 6 hemicenters incompletely fused; and (v) 7th cervical rib long. Other variants and minor defects were noted, but occurred in less than 10% of fetuses. The mean (±SD) score for *manus* ossification was 4.20±0.68, while the mean score for *pes* ossification was 4.14±1.03.

Apparently, no effort was made by the investigators to compare incidences of malformations and variations observed in this study with background levels reported by other investigators in the literature. This would be essential in order to validate this laboratory's methodology especially considering the rarity of some of the findings reported. In addition, the issue of dosing stress (relative to poor dosing technique) was not even considered by the investigators relative to the findings observed. As well, litter data generated do not appear useful for historical control purposes due to the confounding influence of dosing error and unnecessary dosing stress observed in this study.

In conclusion, neither the ability of the testing facility to use the mouse as a test model nor its ability to successfully generate useful historical control data was demonstrated in this study.

The submitted study is classified as **unacceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality and GLP statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Water
   - **Lot/Batch #:** Y04517/015
   - **Purity:** Not provided
   - **Compound Stability:** Not applicable
   - **CAS #of TGAI:** Not applicable

2. **Test animals:**
   - **Species:** Mouse (female)
   - **Strain:** Alpk:AP$_f$CD-1
   - **Age/ mean group weight at study initiation:** Approximately 9 weeks; 38.1 g
   - **Source:** Rodent Breeding Unit, Alderly Park, Cheshire, UK
   - **Housing:** Not provided
   - **Diet:** Pelleted R&M No. 3 diet (source not provided), *ad libitum*
   - **Water:** Tap water *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 22±3°C
     - **Humidity:** 30-70%
     - **Air changes:** At least 15/hr
     - **Photoperiod:** 12 hrs light/12 hrs dark
   - **Acclimation period:** At least 2 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: 9/3/98 End: 11/21/98

2. **Objective:** The objective of this study was to establish standard operating procedures for the staining and evaluation of fetal bone and cartilage, and to provide historical control data to support future developmental toxicity studies in the mouse. Additionally, a dose-range finding study for developmental toxicity (MRID 45641808) was performed to determine an appropriate high dose level of ZA1296 for use in a subsequent dose-range finding study in pregnant mice. A summary is included as an appendix at the end of this DER.

3. **Mating:** Mating was performed at the Rodent Breeding Unit. Females were mated with males overnight (between 8:00 p.m. and 7:00 a.m.). A description of confirmation of mating was not provided; however, the day on which a positive indication of mating was observed was designated as gestation day (GD) 1. Animals were delivered to the performing laboratory on GD 1, 2, or 3.

4. **Animal Assignment:** Animals were randomly assigned to cages according to their GD.

5. **Dosage administration:** Water was administered once daily by oral gavage, on GDs 5-18, in

a volume of 1 mL/100 g of body weight. The gavage volume was adjusted daily on the individual body weights determined prior to gavage. Mice were gavaged at approximately the same time each day.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Mice were checked for mortality, clinical signs, abortions, and premature deliveries within 1 hour of dosing and again towards the end of the day. Detailed observations were recorded daily throughout the study. Body weights were determined on arrival, on GD 3 (if not the day of arrival), on GD 5-18, and prior to sacrifice. Food consumption (g/mouse/day) was measured on GDs 3-5, 5-8, 8-11, 11-14, 14-17, and 17-19. All mice killed at scheduled termination on GD 19 and those found dead or requiring euthanasia during the study were examined macroscopically *post mortem*. Examinations consisted of external observation, examination of the viscera, determination of pregnancy status, enumeration of corpora lutea, gravid uterus weight, number and position of implantations, number of live fetuses, and early and late resorptions. All fetuses were delivered by cesarean section.

2. Fetal Evaluations: All fetuses delivered by cesarean were sexed, weighed, and examined for external abnormalities. All fetuses were dissected and examined for visceral abnormalities, then were eviscerated and fixed in 70% industrial methylated spirits. After approximately 24 hours, the head of each fetus was cut along the fronto-parietal suture line and the brain was examined macroscopically. The carcasses were returned to 70% industrial methylated spirits for subsequent processing and staining with Alizarin Red S and Alcian Blue. The stained fetal skeletons were examined for variation and abnormality of the bone and cartilage, and ossification of the manus and pes was assessed.

D. DATA ANALYSIS

1. Statistical analyses: Data from nonpregnant mice or mice that died intercurrently were not included in calculation of mean body weight or mean food consumption. Only mice with live fetuses were included in calculation of mean litter and fetal data. Statistical analyses were not performed. Data were reported as mean values with standard deviations and number of individuals examined.

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

Pre-implantation loss (%) = (# of corpora lutea - # of implantations)/# of corpora lutea x 100

Post-implantation loss (%) = (# of implantations - # of live fetuses)/# of implantations x 100

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: Ten of the 50 mice did not survive to scheduled termination. One mouse was killed following premature delivery on GD 17, and one mouse died during observation on GD 6. The deaths of the other 8 mice were attributed to poor dosing technique. None of these animals exhibited signs of morbidity prior to the day of death. Two mice were found dead, one each on GD 6 and 13. Six mice were sacrificed due to clinical signs; one on GD 6, two on GD 10, one on GD 11, and two on GD 18. Clinical signs included altered breathing pattern, hunched posture, piloerection, subdued behavior, shaking, closed eyes, cold to touch, and pallor. Since 6 of the 8 deaths attributed to poor dosing technique occurred on or prior to GD 13, the investigators concluded that the dosing technique improved to a some degree during the latter portion of the study.

2. Body weight: Body weight data are summarized in Table 1. The mice were observed to almost double their body weight by GD 19; however, gravid uterus weight accounted for the majority of the increase.

TABLE 1. Mean (±SD) body weights and body weight gains (g)[a]

Interval		Parameter
		Body weight
Pretreatment	Day 3	37.3±2.8
Treatment	Day 5	38.1±2.9
	Day 12	43.2±3.8
	Day 18	61.0±6.9
Post-treatment	Day 19	64.1±8.1
		Body weight gains
Pretreatment	Days 3-5[b]	0.8
Treatment	Days 5-12[b]	5.1
	Days 12-18[b]	17.8
Post-treatment	Days 18-19[b]	3.1
Gravid uterus weight		20.9±6.4
Treatment	Days 5-18[b]	22.9
Corrected[b]		2.0

a Data obtained from pages 22-23 and 27 of the study report (MRID 45651810). n=29
b Calculated by reviewers from data presented in this table

3. Food consumption: Food consumption data are shown in Table 2. Food consumption was observed to increase slightly over the treatment period.

TABLE 2. Mean (±SD) food consumption (g/mouse/day)[a]

Interval		Amount consumed
Pretreatment	Days 3-5	6.7±1.3
Treatment	Days 5-8	7.2±0.7
	Days 8-11	7.3±1.0
	Days 11-14	7.2±0.9
	Days 14-17	8.2±1.2
Post-treatment	Days 17-19	8.9±1.2

a Data obtained from page 24 of the study report (MRID 45651810). n=29

4. Gross pathology: *Post Mortem*/ macroscopic findings were only reported for mice that died prior to scheduled termination. That is, gross findings were only reported for 20% of the animals on study. It was stated that few findings were observed in mice that survived to sacrifice but it appears that no complete necropsies were performed on these animals to support these conclusions. One mouse that survived to sacrifice was observed to have a mass in the thoracic cavity that was adherent to the heart, lungs, thoracic wall, and diaphragm, an enlarged spleen, and the liver was adherent to the diaphragm. These findings were considered to be due to poor dosing technique. In mice that died *in extremis* or were killed prior to study termination, the most common findings, indicative of dosing errors, were adhesions of the lungs (3/10), perforated esophagus (1/10), excess watery fluid in the thoracic cavity (7/10), and adhesions in the thoracic cavity (4/10).

5. Cesarean section data: Cesarean section data is presented in Table 3. One mouse was killed following a premature delivery on GD 17; complete litter resorption was observed in one mouse that survived to termination and at necropsy was found to have signs of misdosing. It was observed that while the overall pregnancy rate for the study was 74% (13 of 50 animals were not pregnant at term), the pregnancy rates for mice delivered to the performing laboratory on GD 1, 2, or 3 were 90.5%, 75.0%, and 46.2%, respectively. Due to this substantial decrease in pregnancy rate, it was concluded by the investigors that the optimal time for shipment of pregnant mice was GD 1. However, many other factors could explain the differences in pregnancy rates.

TABLE 3. Cesarean section observations[a]

Observation	Results
# Animals Assigned (Mated)	50
# Animals Pregnant	37
Pregnancy Rate (%)	74
# Nonpregnant	13
Maternal Wastage	
# Died	10
# Died Pregnant	7
# Died Nonpregnant	3
# Aborted	0
# Premature Delivery	1
Total # Corpora Lutea	Not provided
Corpora Lutea/Dam	16.3±2.4
Total # Implantations	Not provided
(Implantations/Dam)	13.0±3.6
Total # Litters	29
Total # Live Fetuses	Not provided
(Live Fetuses/Dam)	12.0±3.6
Total # Dead Fetuses	Not provided
(Dead Fetuses/Dam)	Not provided
Total # Resorptions[b]	30
Early	21
Late	9
Resorptions/Dam	Not provided
Early	5.9±8.7
Late	2.4±4.4
Litters with Total Resorptions	1
Mean Fetal Weight (g)	1.26±0.10
Males	1.26±0.11
Females	1.24±0.12
Sex Ratio (% Male)	52.6±16.2
Preimplantation Loss (%)	20.6±18.5
Postimplantation Loss (%)	8.3±8.5

a Data obtained from pages 18 and 27-28 of the study report (MRID 45651810).

B. DEVELOPMENTAL TOXICITY

1. External Examination: External findings are presented in Table 4a. Three fetuses were found to have major external defects (malformations). One fetus (0.3% fetuses; 3.4% litters) was found to have extra digits of the forepaw; one fetus was (0.3% fetuses; 3.4% litters) was found to have a malrotated hindlimb; and one fetus (0.3% fetuses; 3.4% litters) was found to have cleft palate. Four fetuses were found to have minor defects. Three fetuses (0.9% fetuses; 6.9% litters) were found to have slight flexion of the hindlimb; one fetus (0.3% fetuses; 3.4% litters) was found to have a slight kink of the tail. No other external findings were observed. No comparisons to background levels in the literature were reported by the investigators.

2. Visceral Examination: Visceral findings are presented in Table 4b. One fetus was found to have a major visceral defect (malformation), missing aortic arch (0.3% fetuses; 3.4% litters).

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Nineteen fetuses were found to have minor defects. Five fetuses (1.4% fetuses; 17.2% litters) were found to have discolored thymus; fourteen fetuses (4.0% fetuses; 37.9% litters) were found to have umbilical artery left of the bladder. No other visceral findings were observed. No comparisons of these findings to background levels reported in the literature were reported by the investigators.

3. Skeletal Examination: Skeletal malformations are presented in Table 4c. Six fetuses were found to have major skeletal defects (malformations). Four of these fetuses (1.1% fetuses; 3.4% litters) came from one litter of 20 fetuses; all exhibited multiple shortened bones in the fore and hindlimbs, including bilateral curved and shortened radius, bilateral shortened ulna, humerus, femur, fibula, tibia, scapula, and ilium. Fused premaxillae and extra metacarpals and misshapen metacarpals were also noted. The remaining 2 fetuses (0.6% fetuses; 3.4% litters) came from a single litter; one fetus was observed to have thoracic arches 7 and 8 fused and cartilage fused between arches 5 and 6, while the other was noted to have multiple minor defects that were collectively classified as a malformation. Considering the suspected rarity of some of these findings, it is surprising that the investigators made no effort to compare these to background levels in the literature. This would be an essential step for validating methodology.

Numerous skeletal variants and minor defects were observed (Table 4d). The variants occurring with the greatest incidence (≥10% of fetuses) were: (i) centrum 3 not ossified (10.3% fetuses; 48.3% litters); (ii) centrum 4 not ossified (11.2% fetuses; 51.7% litters); (iii) centrum 5 not ossified (11.5% fetuses; 44.8% litters); (iv) small holes in sternebrae 6 (31.3% fetuses; 89.7% litters); (v) xiphoid cartilage cleft (27.6% fetuses; 86.2% litters); (vi) 7th cervical rib shortened (44.3% fetuses; 93.1% litters); and (vii) hindpaw calcaneum ossified (19.5% fetuses; 65.5% litters). The minor defects occurring with the greatest incidence (≥10% of fetuses) were: (i) small holes in supraoccipital bones (14.7% fetuses; 69.0% litters); (ii) slight incomplete ossification of the parietal bones (11.5% fetuses; 41.4% litters); (iii) sternebrae 4 incompletely cleft (10.9% fetuses; 55.2% litters); (iv) sternebrae 5 hemicenters incompletely fused (10.3% fetuses; 75.9% litters); (v) sternebrae 6 hemicenters incompletely fused (17.8% fetuses; 72.4% litters); and (vi) 7th cervical rib long (10.1% fetuses; 44.8% litters). Numerous other variants and minor defects were noted, but occurred in less than 10% of fetuses. (No comparisons to the literature were performed by the investigators for validation purposes.)

Finally, an assessment of the ossification of the *manus* (forepaw) and *pes* (hindpaw) was performed. Ossification was graded on a scale from 1 (good) to 6 (poor), and an average score was assigned. The mean (±SD) score for *manus* ossification was 4.20±0.68, while the mean score for *pes* ossification was 4.14±1.03.

TABLE 4a. External findings [% fetuses (% litters)][a]

Observation		
# Fetuses (# litters) examined		348 (29)
Malformations		
Forepaw	extra digits	0.3 (3.4)
Hindlimb	malrotated	0.3 (3.4)
Cleft palate		0.3 (3.4)
Minor defects		
Hindlimb	slight flexion	0.9 (6.9)
Tail	slight kink	0.3 (3.4)

a Data obtained from pages 29, 31, and 32 of the study report (MRID 45651810).

TABLE 4b. Visceral findings [% fetuses (% litters)][a]

Observation		
# Fetuses (# litters) examined		348 (29)
Malformations		
Aortic arch absent		0.3 (3.4)
Minor defects		
Thymus	discolored	1.4 (17.2)
Umbilical artery	left of bladder	4.0 (37.9)

a Data obtained from pages 29, 31, and 32 of the study report (MRID 45651810).

TABLE 4c. Skeletal malformations [% fetuses (% litters)][a]

Observation		
# Fetuses (# litters) examined		348 (29)
Radius	curved	1.1 (3.4)
	shortened	1.1 (3.4)
Ulna	shortened	1.1 (3.4)
Humerus	shortened	1.1 (3.4)
Femur	shortened	1.1 (3.4)
Fibula	shortened	1.1 (3.4)
Tibia	shortened	1.1 (3.4)
Scapula	shortened	1.1 (3.4)
Ilium	shortened	0.6 (3.4)
Skull	premaxilla fused	1.1 (3.4)
Forepaw	extra metacarpals present	0.6 (3.4)
	metacarpals misshapen	0.3 (3.4)
Thoracic arches	arches 7 and 8 fused	0.3 (3.4)
	cartilage fused between arches 5 and 6	0.3 (3.4)
Multiple minor defects		0.3 (3.4%)

a Data obtained from pages 30, 31, and 33-42 of the study report (MRID 45651810).

TABLE 4d. Skeletal variants and minor defects [% fetuses (% litters)][a]

Observation		
# Fetuses (# litters) examined		348 (29)
Variants		
Cervical centra	centrum 3 not ossified	10.3 (48.3)
	centrum 4 not ossified	11.2 (51.7)
	centrum 5 not ossified	11.5 (44.8)
Sternebrae	small holes in sternebrae 6	31.3 (89.7)
Xiphoid	cartilage cleft	27.6 (86.2)
Ribs	cervical rib 7 short	44.3 (93.1)
Hindpaw	calcaneum ossified	19.5 (65.5)
Minor defects		
Skull	small holes in supraoccipital bones	14.7 (69.0)
	parietal bones incompletely ossified	11.5 (41.4)
Sternebrae	sternebrae 4 incompletely cleft	10.9 (55.2)
	sternebrae 5 hemicenters incompletely fused	10.3 (75.9)
	sternebrae 6 hemicenters incompletely fused	17.8 (72.4)
Ribs	cervical rib 7 long	10.1 (44.8)

a Data obtained from pages 30, 31, and 33-42 of the study report (MRID 45651810).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The purpose of this study, which was to ensure that the Central Toxicology Laboratory could achieve compliance with the United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-98-207, August 1998, Health Effects Test Guidelines OPPTS 870.3700, using the mouse as the test species, was successfully accomplished.

B. REVIEWER COMMENTS:

1. Maternal toxicity: Ten of the 50 mice did not survive to scheduled termination. One mouse was killed following premature delivery on GD 17, and one mouse died during observation on GD 6. The deaths of the other 8 mice were attributed to poor dosing technique. Two mice were found dead, one each on GD 6 and 13. Six mice were sacrificed due to clinical signs; one on GD 6, two on GD 10, one on GD 11, and two on GD 18. Clinical signs included altered breathing pattern, hunched posture, piloerection, subdued behavior, shaking, closed eyes, cold to touch, and pallor. Food consumption was observed to increase slightly over the treatment period. The mice were observed to almost double their body weight by GD 19; however, gravid uterus weight accounted for the majority of the increase. Macroscopic findings were only reported for mice that died prior to scheduled termination. It was stated that few findings were observed in mice that survived to sacrifice. One mouse that survived to sacrifice was observed to have a mass in the thoracic cavity that was adherent to the heart, lungs, thoracic wall, and diaphragm, an enlarged spleen, and the liver was adherent to the diaphragm. These findings were considered to be due to poor dosing technique. In mice that died *in extremis* or were killed prior to study

termination, the most common findings, indicative of dosing errors, were adhesions of the lungs (3/10), perforated esophagus (1/10), excess watery fluid in the thoracic cavity (7/10), and adhesions in the thoracic cavity (4/10). Since 6 of the 8 deaths attributed to poor dosing technique occurred on or prior to GD 13, the dosing technique was considered to have improved during the latter portion of the study.

It was observed that while the overall pregnancy rate for the study was 74%, the pregnancy rates for mice delivered to the performing laboratory on GD 1, 2, or 3 were 90.5%, 75.0%, and 46.2%, respectively. Due to this substantial decrease in pregnancy rate, it was concluded by the investigators that the optimal time for shipment of pregnant mice was GD 1.

2. Developmental toxicity:

a. Deaths/Resorptions: One dam was killed following a premature delivery, and complete resorption was observed in another dam that survived to termination and at necropsy was found to have signs of misdosing.

b. Altered Growth: The variants occurring with the greatest incidence (≥10% of fetuses) were: (i) centrum 3 not ossified (10.3% fetuses; 48.3% litters); (ii) centrum 4 not ossified (11.2% fetuses; 51.7% litters); (iii) centrum 5 not ossified (11.5% fetuses; 44.8% litters); (iv) small holes in sternebrae 6 (31.3% fetuses; 89.7% litters); (v) xiphoid cartilage cleft (27.6% fetuses; 86.2% litters); (vi) 7th cervical rib shortened (44.3% fetuses; 93.1% litters); and (vii) hindpaw calcaneum ossified (19.5% fetuses; 65.5% litters). The minor defects occurring with the greatest incidence (≥10% of fetuses) were: (i) small holes in supraoccipital bones (14.7% fetuses; 69.0% litters); (ii) slight incomplete ossification of the parietal bones (11.5% fetuses; 41.4% litters); (iii) sternebrae 4 incompletely cleft (10.9% fetuses; 55.2% litters); (iv) sternebrae 5 hemicenters incompletely fused (10.3% fetuses; 75.9% litters); (v) sternebrae 6 hemicenters incompletely fused (17.8% fetuses; 72.4% litters); and (vi) 7th cervical rib long (10.1% fetuses; 44.8% litters). Numerous other variants and minor defects were noted, but occurred in less than 10% of fetuses. The mean (±SD) score for *manus* ossification was 4.20±0.68, while the mean score for *pes* ossification was 4.14±1.03.

c. Developmental Variations: Four fetuses were found to have minor external defects. Three fetuses (0.9% fetuses; 6.9% litters) were found to have slight flexion of the hindlimb; one fetus (0.3% fetuses; 3.4% litters) was found to have a slight kink of the tail. Nineteen fetuses were found to have minor visceral defects. Five fetuses (1.4% fetuses; 17.2% litters) were found to have discolored thymus; fourteen fetuses (4.0% fetuses; 37.9% litters) were found to have umbilical artery left of the bladder.

d. Malformations: Three fetuses were found to have major external defects. One fetus (0.3% fetuses; 3.4% litters) was found to have extra digits of the forepaw (6 digits on the left, 7 digits on the right); one fetus was (0.3% fetuses; 3.4% litters) was found to have a malrotated hindlimb; and one fetus (0.3% fetuses; 3.4% litters) was found to have cleft palate. One fetus was found to have a major visceral defect, missing aortic arch (0.3% fetuses; 3.4% litters). Six fetuses were found to have major skeletal defects. Four of these fetuses (1.1% fetuses; 3.4% litters) came from one litter of 20 fetuses; all exhibited multiple shortened bones in the fore and hindlimbs,

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including bilateral curved and shortened radius, bilateral shortened ulna, humerus, femur, fibula, tibia, scapula, and ilium. Fused premaxillae and extra metacarpals and misshapen metacarpals were also noted. The remaining 2 fetuses (0.6% fetuses; 3.4% litters) came from a single litter; one fetus was observed to have thoracic arches 7 and 8 fused and cartilage fused between arches 5 and 6, while the other was noted to have multiple minor defects that were collectively classified as a malformation.

Apparently, no effort was made by the investigators to compare incidences of malformations and variations observed in this study with background levels reported by other investigators in the literature. This would be essential in order to validate this laboratory's methodology especially considering the rarity of some of the findings reported. In addition, the issue of dosing stress (relative to poor dosing technique) was not even considered by the investigators relative to the findings observed. As well, litter data generated do not appear useful for historical control purposes due to the confounding influence of dosing error and unnecessary dosing stress observed in this study.

In conclusion, neither the ability of the testing facility to use the mouse as a test model nor its ability to successfully generate useful historical control data was demonstrated in this study.

The submitted study is classified as **acceptable/non-guideline**.

C. STUDY DEFICIENCIES: This is a non-guideline study. The study investigators were not successful in reaching their objectives.

Since this is a range finding study, only a summary is provided.

In a non-guideline dose range finding study (MRID 45651808), ZA1296 (Mesotrione; 96.8% a.i.; Lot/Batch # P17) in water was administered daily by oral gavage at a dose volume of 10 mL/kg body weight to 5 Alpk:AP$_f$CD-1 female mice/group at dose levels of 0, 600, 800, or 1000 mg/kg for 8 consecutive days. The objective of this study was to investigate the effects of oral (gavage) administration of ZA1296 on female Alpk:AP$_f$CD-1 mice to determine an appropriate high dose level for use in a subsequent dose finding study in pregnant mice of the same strain. Additionally, plasma tyrosine levels were determined in the 600 and 800 mg/kg animals at two hour intervals up to 8 hours post-dosing.

No effects of treatment were observed on clinical observations, body weight, or food consumption. Mean body weights of all test groups exceeded controls levels at all measured levels. As well, food consumption generally exceeded control levels in all test groups at nearly every interval measured. Patterns of food consumption increased/decreased consistently and similarly over time in all dosed groups and controls suggesting methodology issues/problems may be present.

Two 1000 mg/kg animals were found dead on Day 4; 1 animal was found dead prior to dosing, and the other was found dead approximately 8 minutes after dosing. One 800 mg/kg animal was found dead on Day 8, approximately 6 hours after dosing. Relative to these deaths, the investigators stated,"No reasons for their death could be established but the cause of death was considered to be treatment related." However, all animals were discarded without necropsy. Hence, there is no reason to support the conclusion that the deaths were related to administration of the test material. Deaths may also have been related to dosing error and NOT the test material. Note that numerous problems with dosing which resulted in the deaths of animals were reported in MRID 45651810).

Plasma tyrosine values for the 600 and 800 mg/kg groups were similar in magnitude at each time point. Plasma tyrosine levels increased approximately 3-fold from 0-2 hours post-dosing, appeared to peak from 4-6 hours post-dosing, and began to decline by 8-hours post-dosing.

The maximum dose level to be used in a subsequent developmental toxicity study in the mouse was NOT determined in this study.

This range-finding study is classified as un**acceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Flagging statements were provided.

(6)

DATA EVALUATION RECORD

ZA1296 (MESOTRIONE)

Study Type: Non-guideline Study; Investigation of Liver and Kidney Enzyme Parameters in Control Rat Pups

Work Assignment No. 1-01-16 D (MRID 45651809)

Prepared for
Health Effects Division
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Laurence D.Chitlik, D.A.B.T. **Signature:** _____
Toxicology Branch 1, Health Effects Division (7509C) **Date** _____
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050845

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline Study; Investigation of Liver and Kidney Enzyme Parameters - Rats.

PC CODE: 122990 **DP BARCODE:** D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): Untreated

SYNONYMS: None

CITATION: Moxon, M.E. (2000) Investigation of liver and kidney enzyme parameters in control rat pups from new born to age 42 days. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL Study No. RR0798, Syngenta No. 1253-98, November 16, 2000. MRID 45651809. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC.

EXECUTIVE SUMMARY: In a non-guideline study (MRID 45651809), 36 untreated time-mated pregnant Alpk:AP,SD female rats were received on gestation day (GD) 1 and allowed to litter normally. Three females and their litters were killed on postnatal days (PND) 1, 2, 3, 4, 5, 8, 12, 15, 22, 29, 35, and 42. These animals were exsanguinated, and the liver and kidneys were removed. The levels of plasma tyrosine, and liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities were determined. The purpose of this study was to provide a database on the basal levels of plasma tyrosine and liver and kidney TAT and HPPD in control rat pups up to 42 days of age.

Maternal plasma tyrosine levels appeared to rise from 64.6 nmol/mL on PND 1 to 105.6 nmol/mL on PND 29. Thereafter, the levels reduced to the end of the sampling period on day 42. Maternal liver TAT activity fluctuated over the course of the sampling period without any apparent pattern over time. A low value was observed on PND 4 (0.211 nmol HPPA/min/mg protein) perhaps due to experimental error. Maternal kidney TAT levels were approximately 4-fold lower than liver levels. Kidney TAT activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time.

Maternal Liver HPPD activity appeared to fluctuate over the course of the sampling period

without any apparent pattern over time. Kidney HPPD activity also varied over the sampling period but generally was half to two-thirds of the liver levels. In addition, although kidney HPPD activity appeared quite variable, the range of fluctuation was smaller than observed in the liver.

Pup plasma tyrosine levels rose from birth, peaking on PND 8-12 for both sexes. Plasma tyrosine levels then abruptly fell on PND 22 to approximately the same level as observed in adult females. Pup plasma levels remained lower but variable over the remainder of the sampling period.
Liver TAT activity appeared to fluctuate throughout the study, with male and female offspring (4.943-5.444 nmol HPPA/min/mg protein vs 3.347 nmol HPPA/min/mg protein) having approximately the same activity as adult females. Kidney TAT levels were approximately 7-fold lower than liver levels. Kidney TAT activity also appeared to fluctuate throughout the study, with male and female offspring (0.696-0.798 nmol HPPA/min/mg protein vs 0.797 nmol HPPA/min/mg protein) having approximately the same range of activity as in adult females.

Pup liver HPPD activity (in both sexes) generally rose from a low on PND 1 (0.484-0.625 µL O_2/min/mg protein), and after significant fluctuation increased markedly after PND 15 exceeding adult female levels by PND 22. Thereafter, levels fluctuated (although at increased levels) for the remainder of the study. Kidney HPPD levels were approximately 3-fold lower than liver levels. Kidney HPPD activity rose from a low on PND 1 (0.187-0.194 µL O_2/min/mg protein) and approached adult female levels by PND 22. Activity fluctuated for the entire sampling period of the study.No apparent differences between sexes were observed up to PND 42 in plasma tyrosine levels or liver and kidney TAT and HPPD activities.

It is unclear how (or if) problems with analytical methodology might have contributed to the variability of activity for tyrosine, liver and kidney TAT and HPPD activities in this study. As well, small sample size may have contributed to the variability of activity observed at different sampling intervals. In addition, without replicate analyses, it is difficult to assess the utility of these data as background levels for this test species.

The submitted study is classified as **acceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality and GLP statements were provided. It was stated that this study was not conducted in compliance with any GLP regulations (i.e., US EPA 40 CFR Parts 160 and 792) and was not audited by the QA unit of the performing lab; however, it was conducted to the highest standards of research practices.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: None

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat (female)	
Strain:	Alpk:AP$_f$SD	
Age/weight at arrival:	10-12 weeks; 220-300 g	
Source:	Rodent Breeding Unit, Alderly Park, Cheshire, UK	
Housing:	Females were individually housed with litters until pups were 29 days old. Pups were then rehoused as litter mates, up to 5/cage.	
Diet:	Powdered CT1 diet (Special Diet Services, Ltd., Witham, Essex, UK), *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	At least 15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	None	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: 7/28/98 End: 9/29/98

2. Objective: The objective of this study was to provide a database on the basal levels of plasma tyrosine and activities of liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) in control rat pups up to postnatal day (PND) 42.

3. Mating: Mating was performed by the supplier. A description of mating was not provided; however, the day on which a positive indication of mating was observed (sperm detected in a vaginal smear) was designated as gestation day (GD) 1. Animals were delivered to the performing laboratory on GD 1.

4. Study design: Thirty-six pregnant rats were placed on study upon receipt by the performing laboratory (GD 1). Dams were allowed to litter normally. Three dams and their litters were sacrificed at each time point (PND 1, 2, 3, 4, 5, 8, 12, 15, 22, 29, 35, and 42). Plasma tyrosine levels and liver and kidney TAT and HPPD activities were measured in the dams and the pups.

5. Dosage preparation, administration, and analysis: Not applicable

C. METHODS

1. Maternal evaluations: Rats were checked for mortality and clinical signs of toxicity daily during the study. Detailed observations were recorded when the animals were weighed. Body weights were determined on GD 1, 8, 15, and 22; on PND 1, 5, 8, 12, 15, 22, and 29; and at termination. Food consumption was recorded weekly during gestation. Three females and their litters were killed on PND 1, 2, 3, 4, 5, 8, 12, 15, 22, 29, 35, and 42. Females were killed by overexposure to halothane anesthesia and exsanguinated by cardiac puncture, and the liver and kidneys were removed.

2. Pup evaluations: Each litter was examined daily for dead or abnormal pups; these were discarded without examination. Detailed observations were recorded when the pups were weighed. A count of all pups (live and dead) was made within 24 hours of parturition and on PND 5, 8, 12, 15, 22, and 29. Body weights were determined on PND 1, 5, 8, 12, 15, 22, 29, 35, and 42, and at termination. Food consumption was recorded weekly following weaning. On the day of termination, the litter with the most pups was killed first; and blood, liver, and kidneys were taken from each animal. In the second and third litters, blood was taken from all pups; liver and kidneys were taken from a maximum of 4 pups/sex/litter. Any runts in the 2nd and 3rd litters were not used for tissue collection. Pups were killed by cervical dislocation and exsanguinated by decapitation through PND 8; thereafter, pups were killed by overexposure to halothane anesthesia followed by cardiac puncture.

3. Plasma tyrosine and tissue enzyme activity analysis: Blood samples were pooled for all of the pups of each sex per litter. All blood samples were centrifuged, and the plasma was removed and stored at -20°C until analysis. Plasma tyrosine levels were measured by HPLC with UV detection using a Hichrom S5ODS2 column with a mobile phase gradient of 100% acetonitrile mixing with water:acetonitrile:trifluoroacetic acid (950:50:2; v/v). Livers and kidneys were weighed, homogenized, and centrifuged to yield cytosols that were then aliquoted and stored at -70°C until enzyme analysis was performed. Tyrosine aminotransferase (TAT) catalyzes the conversion of tyrosine to *p*-hydroxyphenylpyruvic acid (HPPA). HPPA will react with phenazine methosulphate to form a colored reaction product that can be measured by a spectrophotometer at 675 nm. Cytosols were analyzed spectrophotometrically (in duplicate) for TAT activity. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) catalyzes the oxidative decarboxylation and rearrangement of HPPA to homogentisate, with incorporation of both atoms of molecular oxygen into the product. Thus, HPPD activity in solution can be measured by monitoring oxygen consumption. Cytosolic oxygen consumption was measured using an oxygen electrode.

D. DATA ANALYSIS: All data were presented as mean values ±SD. As only control animals were evaluated, no statistical analyses were performed.

II. RESULTS

A. MATERNAL EVALUATIONS: It was stated that no significant clinical signs were

observed. Individual body weight data were included but not tabulated, and food consumption data was not provided; however, these data did not contribute to the stated objective of this study.

1. Plasma tyrosine analysis: Maternal plasma tyrosine levels are summarized in Table 1. Plasma tyrosine levels appeared to rise from 64.6 nmol/mL on PND 1 to 105.6 nmol/mL on PND 29. Thereafter, the levels reduced to the end of the sampling period on day 42.

Table 1. Plasma tyrosine levels (nmol/mL; mean±SD) in maternal rats following parturition.[a]

Day of sacrifice	Tyrosine
PND 1	64.6±9.2
PND 2	68.3±13.4
PND 3	80.7±6.5
PND 4	83.7±12.2
PND 5	79.6±7.8
PND 8	83.2±16.2
PND 12	81.4±15.2
PND 15	83.5±6.3
PND 22	90.2±28.2
PND 29	105.6±16.2
PND 35	83.4±15.5
PND 42	77.0±6.2
Overall mean[b]	81.8

a Data obtained from page 51 of the study report.
b Calculated by reviewers from data presented in this table

2. TAT activity: Maternal TAT activity in liver and kidney cytosols are summarized in Table 2. Liver TAT activity fluctuated over the course of the sampling period without any apparent pattern over time. A low value was observed on PND 4 (0.211 nmol HPPA/min/mg protein). The investigators claimed this must have been experimental error. Kidney TAT levels were approximately 4-fold lower than liver levels. Kidney TAT activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time.

Table 2. TAT activity (nmol HPPA/min/mg protein: mean±SD) in liver and kidney cytosols of maternal rats following parturition.[a]

Day of sacrifice	Liver	Kidney
PND 1	2.826±1.430	1.105±0.471
PND 2	2.694±0.846	0.613±0.426
PND 3	3.451±0.351	1.140±0.850
PND 4	0.211±0.283[b]	1.079±0.573
PND 5	2.890±0.116	0.628±0.034
PND 8	3.391±0.546	0.371±0.203
PND 12	3.828±0.798	0.853±0.362
PND 15	4.206±0.891	0.923±0.127
PND 22	3.621±0.321	0.806±0.123
PND 29	2.468±0.204	0.538±0.289
PND 35	3.399±0.588	0.682±0.267
PND 42	4.048±1.393	0.821±0.297
Overall mean[c]	3.347	0.797

a Data obtained from pages 67 and 79 of the study report.
b Not included in calculation of overall mean
c Calculated by reviewers from data presented in this table

3. <u>4-Hydroxyphenylpyruvate dioxygenase (HPPD) activity</u>: Maternal HPPD activity in liver and kidney cytosols are summarized in Table 3. Liver HPPD activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time. Kidney HPPD activity also varied over the sampling period but generally was half to two-thirds of the liver levels. In addition, although kidney HPPD activity appeared quite variable, the range of fluctuation was smaller than for the liver.

Table 3. HPPD activity (μL O_2/min/mg protein: mean±SD) in liver and kidney cytosols of maternal rats following parturition.[a]

Day of sacrifice	Liver	Kidney
PND 1	0.813±0.105	0.766±0.102
PND 2	0.953±0.092	0.627±0.132
PND 3	1.217±0.098	0.893±0.029
PND 4	1.447±0.221	0.807±0.053
PND 5	1.179±0.140	0.670±0.168
PND 8	1.380±0.302	0.703±0.261
PND 12	1.564±0.145	0.843±0.038
PND 15	0.675±0.476	0.564±0.077
PND 22	1.724±0.197	0.537±0.057
PND 29	1.405±0.309	0.434±0.037
PND 35	1.215±0.192	0.448±0.033
PND 42	1.860±0.056	0.547±0.059
Overall mean[b]	1.286	0.653

a Data obtained from pages 85 and 96 of the study report.
b Calculated by reviewers from data presented in this table

B. PUP EVALUATIONS: No significant clinical signs were observed, and the data were not included in the study report. Individual litter data (body weight, sex, and number of pups) were included but not tabulated, and food consumption data was not provided; however, these data did not contribute to the stated objective of this study.

1. Plasma tyrosine analysis: Pup plasma tyrosine levels are summarized in Table 4. Plasma tyrosine levels rose from birth, peaking on PND 8-12 for both sexes. Plasma tyrosine levels then abruptly fell on PND 22 to approximately the same level as observed in adult females. Pup plasma levels remained lower but variable over the remainder of the sampling period.

Table 4. Plasma tyrosine levels (nmol/mL; mean±SD) in male and female rats for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	160.5±26.2	173.7±74.9
PND 2	83.8±12.0	90.9±32.7
PND 3	143.7±22.7	132.3±18.5
PND 4	165.0±38.4	165.0±29.1
PND 5	181.0±31.6	189.0±23.6
PND 8	253.3±64.3	238.3±48.2
PND 12	233.3±25.0	278.4±36.6
PND 15	234.4±34.0	217.3±36.7
PND 22	91.4±20.1	88.7±22.8
PND 29	111.8±8.7	88.7±14.0
PND 35	108.1±9.1	95.7±9.2
PND 42	104.2±7.4	82.2±6.7
Overall[b]	155.9	153.3

a Calculated by reviewers from data obtained from pages 60-62 of the study report. (See attached spreadsheet 45651809)

b Calculated by reviewers from data presented in this table

2. <u>Tyrosine aminotransferase (TAT) activity</u>: Pup TAT activity is shown in Tables 5a and b. Liver TAT activity appeared to fluctuate throughout the study, with male and female offspring (4.943-5.444 nmol HPPA/min/mg protein vs 3.347 nmol HPPA/min/mg protein) having approximately the same activity as adult females. Kidney TAT levels were approximately 7-fold lower than liver levels. Kidney TAT activity also appeared to fluctuate throughout the study, with male and female offspring (0.696-0.798 nmol HPPA/min/mg protein vs 0.797 nmol HPPA/min/mg protein) having approximately the same range of activity as in adult females.

Table 5a. TAT activity (nmol HPPA/min/mg protein; mean±SD) in liver cytosols of male and female rats for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	7.890±6.664	8.451±6.926
PND 2	6.289±3.652	5.936±2.979
PND 3	6.605±4.634	7.101±3.978
PND 4	2.062±1.177	3.141±1.853
PND 5	5.587±2.189	6.823±1.912
PND 8	3.394±0.951	4.525±1.798
PND 12	4.382±1.016	4.254±1.640
PND 15	7.532±2.147	8.829±2.995
PND 22	6.203±1.151	6.305±2.225
PND 29	3.085±0.491	2.962±0.602
PND 35	3.097±0.678	3.417±0.610
PND 42	3.193±0.689	3.582±1.283
Overall[b]	4.943	5.444

a Calculated by reviewers from data obtained from pages 78 and 80 of the study report. (See attached spreadsheet 45651809)

b *Calculated by reviewers from data presented in this table*

Table 5b. TAT activity (nmol HPPA/min/mg protein; mean±SD) in kidney cytosols of male and female rats for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	1.646±0.748	0.885±0.122
PND 2	0.677±0.219	0.809±0.134
PND 3	0.965±0.538	0.401±0.298
PND 4	0.807±0.289	1.277±0.560
PND 5	0.814±0.590	0.719±0.408
PND 8	0.327±0.275	1.656±1.804
PND 12	0.679±0.542	0.716±0.390
PND 15	0.460±0.287	0.621±0.200
PND 22	0.682±0.483	0.585±0.216
PND 29	0.339±0.205	0.700±0.609
PND 35	0.399±0.146	0.600±0.212
PND 42	0.560±0.106	0.609±0.285
Overall[b]	0.696	0.798

a Data obtained from page 78 of the study report.

b Calculated by reviewers from data presented in this table

3. <u>4-Hydroxyphenylpyruvate dioxygenase (HPPD) activity</u>: Pup HPPD activity is shown in Tables 6a and b. Liver HPPD activity (in both sexes) generally rose from a low on PND 1 (0.484-0.625 µL O_2/min/mg protein), and after significant fluctuation increased markedly after PND 15 exceeding adult female levels by PND 22. Thereafter, levels fluctuated (although at increased levels) for the remainder of the study. Kidney HPPD levels were approximately 3-fold lower than liver levels. Kidney HPPD activity rose from a low on PND 1 (0.187-0.194 µL O_2/min/mg protein) and approached adult female levels by PND 22. Activity fluctuated for the entire sampling period of the study.

Table 6a. HPPD activity (µL O_2/min/mg protein; mean±SD) in liver cytosols of male and female rats for PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	0.625±0.122	0.484±0.088
PND 2	0.663±0.093	0.664±0.094
PND 3	0.788±0.085	0.728±0.065
PND 4	0.708±0.165	0.766±0.070
PND 5	0.594±0.185	0.566±0.133
PND 8	0.836±0.266	0.897±0.159
PND 12	0.793±0.158	0.678±0.124
PND 15	0.359±0.214	0.260±0.096
PND 22	2.149±0.449	2.115±0.352
PND 29	2.120±0.246	2.239±0.304
PND 35	1.525±0.124	1.757±0.183
PND 42	1.629±0.110	2.047±0.374
Overall[b]	1.066	1.100

a Calculated by reviewers from data obtained from page 97 of the study report. (See attached speadsheet 45651809)

b Calculated by reviewers from data presented in this table

Table 6b. HPPD activity (μL O_2/min/mg protein; mean±SD) in kidney cytosols of male and female rats from PND 1-42.[a]

Day of sacrifice	Males	Females
PND 1	0.194±0.027	0.187±0.106
PND 2	0.198±0.027	0.175±0.104
PND 3	0.251±0.049	0.219±0.028
PND 4	0.246±0.115	0.251±0.052
PND 5	0.225±0.097	0.316±0.173
PND 8	0.189±0.073	0.185±0.068
PND 12	0.228±0.087	0.299±0.119
PND 15	0.264±0.043	0.267±0.022
PND 22	0.506±0.105	0.519±0.114
PND 29	0.578±0.075	0.649±0.078
PND 35	0.446±0.093	0.544±0.065
PND 42	0.394±0.071	0.703±0.162
Overall[b]	0.310	0.360

a Data obtained from page 95 of the study report.
b Calculated by reviewers from data presented in this table

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The results of this study confirm the findings of a previous study and demonstrate that TAT levels increase markedly after birth, and then decline to adult levels by day 22. In the neonatal rat pup, there were no differences in male and female TAT activity. This is in contrast to the marked sex difference observed in rats studied at the end of a 90-day toxicity study (CTL reports number CTL/R/1304 and CTL/R/1315). Therefore, between day 22 and day 150 (age at the end of 90-day study) a divergence of TAT levels occurs between male and female rats. Similarly by day 22, the levels of HPPD and plasma tyrosine have achieved adult levels and as with TAT, and apparently,the sex difference in HPPD only becomes apparent after sexual maturity.

B. REVIEWER COMMENTS:

Maternal plasma tyrosine levels appeared to rise from 64.6 nmol/mL on PND 1 to 105.6 nmol/mL on PND 29. Thereafter, the levels reduced to the end of the sampling period on day 42. Maternal liver TAT activity fluctuated over the course of the sampling period without any apparent pattern over time. A low value was observed on PND 4 (0.211 nmol HPPA/min/mg protein) perhaps due to experimental error. Maternal kidney TAT levels were approximately 4-fold lower than liver levels. Kidney TAT activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time.

Maternal Liver HPPD activity appeared to fluctuate over the course of the sampling period without any apparent pattern over time. Kidney HPPD activity also varied over the sampling period but generally was half to two-thirds of the liver levels. In addition, although kidney HPPD activity appeared quite variable, the range of fluctuation was smaller than observed in the liver.

Pup plasma tyrosine levels rose from birth, peaking on PND 8-12 for both sexes. Plasma tyrosine levels then abruptly fell on PND 22 to approximately the same level as observed in adult females. Pup plasma levels remained lower but variable over the remainder of the sampling period.
Liver TAT activity appeared to fluctuate throughout the study, with male and female offspring (4.943-5.444 nmol HPPA/min/mg protein vs 3.347 nmol HPPA/min/mg protein) having approximately the same activity as adult females. Kidney TAT levels were approximately 7-fold lower than liver levels. Kidney TAT activity also appeared to fluctuate throughout the study, with male and female offspring (0.696-0.798 nmol HPPA/min/mg protein vs 0.797 nmol HPPA/min/mg protein) having approximately the same range of activity as in adult females.

Pup liver HPPD activity (in both sexes) generally rose from a low on PND 1 (0.484-0.625 µL O_2/min/mg protein), and after significant fluctuation increased markedly after PND 15 exceeding adult female levels by PND 22. Thereafter, levels fluctuated (although at increased levels) for the remainder of the study. Kidney HPPD levels were approximately 3-fold lower than liver levels. Kidney HPPD activity rose from a low on PND 1 (0.187-0.194 µL O_2/min/mg protein) and approached adult female levels by PND 22. Activity fluctuated for the entire sampling period of the study.No apparent differences between sexes were observed up to PND 42 in plasma tyrosine levels or liver and kidney TAT and HPPD activities.

It is unclear how problems with analytical methodology might have contributed to the variability of activity for tyrosine, liver and kidney TAT and HPPD activities in this study. As well, small sample size may have contributed to the variability of activity observed at different sampling intervals. In addition, without replicate analyses, it is difficult to assess the utility of these data as background levels for this test species.

The submitted study is classified as **acceptable/non-guideline**.

C. <u>STUDY DEFICIENCIES</u>: This is a non-guideline study. Activities reported are more variable than desired. No assessment by the investigators was included relative to analytical methodologies utilized in this study. As well, perhaps the utility of the study would have been enhanced had the sampling period been extended until the animals reached sexual maturity.

DATA EVALUATION RECORD

ZA1296 (MESOTRIONE)

Study Type: Non-guideline Study; Investigation of the Effects of ZA1296 and Tyrosine on Developmental Toxicity in the Rabbit

Work Assignment No. 1-01-16 G (MRID 45651812)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Laurence D. Chitlik, D.A.B.T. **Signature:** _____
Toxicology Branch , Health Effects Division (7509C) **Date** _____
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050845

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline Study; Investigation of the Effects of ZA1296 and Tyrosine on Developmental Toxicity in the Rabbit.

PC CODE: 122990 **DP BARCODE:** D295934
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): ZA1296 technical (Mesotrione; 96.8% a.i.)

SYNONYMS: 2-[4-(Methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Moxon, M.E. (2000) Investigation of the effects of ZA1296 and tyrosine on developmental toxicity in the rabbit. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study Id.: CTL Study No. RB0802, Syngenta No. 1281-99, April 13, 2000. MRID 45651812. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC.

EXECUTIVE SUMMARY: In a non-guideline developmental toxicity study (MRID 45651812), ZA1296 (Mesotrione; Lot/batch # P17; 96.8% a.i.) was administered in water daily by oral gavage at a dose volume of 10 mL/kg body weight to 20 New Zealand White female rabbits/group at dose levels of 0 or 500 mg/kg on gestation days (GD) 8 through 20. Additionally, rabbits were fed either diet containing 1% tyrosine or control diet during this period, such that there were 4 experimental groups total (2 x 2 factorial design): i) water gavage + control diet (group 1); ii) water gavage + 1% tyrosine diet (group 2); iii) 500 mg/kg ZA 1296 gavage + control diet (group 3); and iv) 500 mg/kg ZA1296 gavage + 1% tyrosine diet (group 4). All does were sacrificed on GD 30, and their fetuses were removed and examined. Maternal plasma tyrosine levels and liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities were determined. The objective of this study was to manipulate maternal plasma tyrosine levels in order to investigate the effects of tyrosinaemia on fetal skeletal ossification, and to determine if treatment with ZA1296 caused increased incidence of abortion.

Maternal Toxicity:

There were no effects of treatment on maternal survival, clinical signs, ophthalmoscopic

examination, or gross pathology reported . Maternal body weights (adjusted for initial weight) were decreased ($p \leq 0.05$) in the group 4 (500 mg/kg ZA1296 + 1% tyrosine) does during GD 14-21 (↓1-3%), and remained decreased (not significant) through GD 30 (↓2%). Overall (GD 4-30) body weight gains were also decreased in the group 4 does (↓7%) compared to controls. However, in the rabbit both the small body weight and body weight gain changes as observed in this study are considered normal variation, minor and not biologically significant and do not constitute maternal toxicity. As well, food consumption was decreased ($p \leq 0.05$) in the group 4 does by 27% on GD 8-11 and continued throughout the remainder of the dosing period (GD 11-21), although without statistical significance (↓8-22%). Additionally, the group 3 (500 mg/kg ZA1296 only) does were observed to have decreased food consumption throughout dosing (GD 8-21; ↓7-13%; not significant). In both cases, food consumption returned to control levels by GD 24-27. Rabbits are notorious for spillage of feed and differences like these in the absence of clear body weight changes are not considered biologically relevant.

Plasma tyrosine levels were increased ($p \leq 0.01$) in all groups in a step-wise fashion, with the greatest increases occurring in the group 4 does (↑284-1773%). Levels peaked in all groups at 12 hours after treatment with ZA1296, and returned to maintenance levels at 24 hours post-dosing. Kidney TAT activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓42-58%) compared to controls. Liver HPPD activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓54-57%). Kidney HPPD activity was decreased ($p \leq 0.01$) in the group 3 and 4 does (↓69-75%). No clinical signs or pathology were associated with these findings. Therefore, clear indications of maternal toxicity were not observed in this study.

Developmental Toxicity:

One group 4 doe was killed on GD 22 following the abortion of several fetuses. This animal had demonstrated negligible food consumption from GD 17 and a loss of body weight from GD 19. Necropsy revealed a flaccid heart with pale areas on the ventricles; however, this finding was not considered to be treatment-related. No effects of treatment were observed on numbers of live fetuses, resorptions (early or late) or post-implantation loss.

No effects on fetal body weight were apparent. Increased ($p \leq 0.05$) incidences of the following skeletal defects were noted: incomplete ossification of the odontoid of the cervical centra in groups 3 (13.6% fetuses; 38.9% litters) and 4 (14.5% fetuses; 52.9% litters), and incompletely ossified pubis in group 4 (6.9% fetuses; 47.1% litters). The proportion of animals having a manus score of 3 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a manus score of 4. Similarly, the proportion of animals having a pes score of 1 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a pes score of 2.

Increased incidence ($p \leq 0.05$) of extra vessel(s) arising from the aortic arch was observed in groups 3 (4.8% fetuses; 27.8% litters) and 4 (6.9% fetuses; 29.4% litters). Enlarged ventricle and reduced ventricle of the heart were observed in group 2 (1% tyrosine only) fetuses (0.8% fetuses; 7.1% litters) and group 4 (1.4% fetuses; 11.8% litters) fetuses. Increased ($p \leq 0.05$) incidences of

the following findings were observed: long thoracolumbar rib 13 in groups 2 (35.2% fetuses; 92.9% litters), 3 (73.5% fetuses; 88.9% controls), and 4 (92.4% fetuses; 100% litters), and 27 pre-pelvic bilateral vertebrae in groups 3 (49.7% fetuses; 88.9% litters) and 4 (86.2% fetuses; 100% litters). Extreme constriction of the pulmonary artery was noted in groups 3 (0.7% fetuses; 5.6% litters) and 4 (1.4% fetuses; 11.8% litters), and extreme dilation of the aorta was observed in groups 2 (0.8% fetuses; 7.1% litters), 3 (1.4% fetuses; 11.1% litters), and 4 (2.1% fetuses; 11.8% litters).

This is not a guideline study and due to study design, no dose response assessment was possible; a NOAEL and LOAEL were not determined. **In conclusion, in the absence of maternal toxicity, developmental toxicity was observed in this study with ZA1296 (Mesotrione, 96.8% a.i.) at a dose level of 500mg/kg (gavage) with either 1% or 0% tyrosine mixed in the diet.**

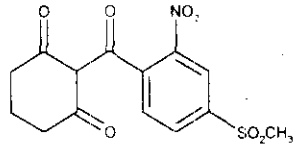
The submitted study is classified as **acceptable/non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ZA1296 technical (Mesotrione)

Description:	Light beige solid
Lot/Batch #:	P17
Purity:	96.8% a.i.
Compound stability:	Not provided
CAS #of TGAI:	104206-82-8
Structure:	

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White	
Age/ weight range at arrival:	At least 16 weeks; 2.9-4.3 kg	
Source:	Harlan Interfauna (UK), Ltd.	
Housing:	Rabbits were individually housed in mobile rabbit units.	
Diet:	Harlan Teklad TRB Rabbit diet 9603 *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	17±3°C
	Humidity:	30-70%
	Air changes:	At least 15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	5-6 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: 4/15/99 End: 7/19/99

2. Objective: In a previous developmental toxicity study (CTL report number CTL/P/4892), dose levels of up to 500 mg/kg/day ZA1296 were associated with changes in ossification of the fetal skeleton and increased incidence of abortion, although this finding was not conclusively linked to treatment. Therefore, the objective of this study was to manipulate maternal plasma tyrosine levels in order to investigate the effects of tyrosinaemia on fetal skeletal ossification, and to determine if treatment with ZA1296 caused increased incidence of abortion.

3. Mating: Nulliparous, nonpregnant females were mated by the supplier. A description of the mating procedure was not provided; however, the day on which mating occurred was designated

as gestation day (GD) 1. Does were delivered to the performing laboratory on GD 2 or 3.

4. Study design and animal assignment: Treatment-related effects of the test substance, tyrosine, and the interaction of the test substance with a single concentration of tyrosine were examined in a 2 x 2 factorial study design. Pregnant females were randomly assigned (blocked by location within the experimental array) to the test groups shown in Table 1. Additionally, sisters and females which had been mated with the same male were distributed across the groups.

Table 1. *Animal assignment*[a]

Test group	ZA1296 (mg/kg/day)	Dietary tyrosine concentration (%)	# of females
1	0	0.0	20
2	0	1.0	20
3	500	0.0	20
4	500	1.0	20

a Data obtained from page 18 of the study report.

5. Dose selection rationale: It was stated that the dose of 500 mg/kg ZA1296 was selected as it was the highest dose level used in a previous study, and that the dietary level of tyrosine (1.0%) was selected based on previous studies conducted by the performing laboratory. No further information was provided.

6. Dosage and diet preparation: An appropriate amount of vehicle was added to a weighed amount of ZA1296 (adjusted for purity) and thoroughly mixed. Seven preparations were made per group, and each preparation was subdivided into aliquots after mixing. A fresh aliquot was used for each day of the study. Dose preparations were stored at room temperature. An appropriate amount of tyrosine was mixed with finely ground diet to make a premix. The premix was then diluted with an appropriate amount of finely ground diet to yield 60 kg of diet containing a nominal concentration of 1% tyrosine. The diet was then formed into 3 mm pellets for feeding. Neither the ZA1296 dose preparations nor the diet batches were analyzed for homogeneity, concentration, or stability.

7. Dosage and diet administration: Females were gavaged with one of 2 doses containing ZA1296 (0 or 500 mg/kg) and were fed diets containing tyrosine (0 or 1.0%) from GD 8-20. The does were dosed each day with ZA1296 in a dose volume of 10 mL/kg body weight; animals that did not receive ZA1296 were gavaged with water. Dose volumes were adjusted daily according to individual body weight. Dosing was sequential, in group order, and performed at approximately the same time each day. Adequate mixing was accomplished by shaking the dose preparations prior to and during dosing. Diet containing 0 or 1.0% tyrosine was given to the does on the morning of GD 8 until the morning of GD 21. Diet without additional tyrosine was given before and after this period.

C. METHODS

1. Maternal observations and evaluations: Maternal clinical observations, food consumption, body weights, ophthalmoscopic observations, and plasma tyrosine levels were measured during the study; liver and kidney tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) activities were determined after sacrifice. Cage-side observations for mortality and clinical signs of toxicity were made as soon as possible after dosing and towards the end of each work day. Detailed clinical observations were recorded daily at the same time as body weight measurement, where appropriate. Body weights were recorded on arrival, GD 4, 8-21, 24, 27, and 30. Food consumption (g/rabbit/day) was determined for GD 4-8, 8-11, 11-14, 14-17, 17-21, 21-24, 24-27, and 27-30. Blood samples were collected from the marginal ear vein prior to dosing and 12 and 24 hours after dosing on GD 8, 14, and 20; a single blood sample was also collected on GD 29. Blood samples were centrifuged, and the plasma removed and stored at -18°C until tyrosine analysis was performed. Urine samples were collected from each animal during the pre-dosing period, and from selected rabbits (based on plasma tyrosine levels) on GD 18 and at termination. Rabbits were placed into a metabolism cage until a sample was obtained, and feed and water were available *ad libitum* during collection. Urine samples were analyzed for phenolic acids and tyrosine metabolites, but the data were considered to be of little value in achieving the overall study goal and were not included in the study report. Ophthalmoscopic examinations were performed on GD 4, 5, or 6 and on GD 29 using an indirect ophthalmoscope with pupillary dilation.

2. Postmortem observations:

a. Maternal: Rabbits sacrificed at study termination (GD 30) and those killed *in extremis* or following abortion were killed by an intravenous injection of an overdose of pentobarbitone sodium. All does, including those found dead, were subjected to a necropsy. The liver and kidneys were removed (except from animals found dead), and samples were analyzed for TAT and HPPD activity. The gravid uterus was removed and weighed (only from animals at scheduled termination), and the number of corpora lutea, number and position of implantations, number of live fetuses, and number of early and late resorptions were determined.

b. Litter: The fetuses were delivered by cesarean section; the weight, sex, and external, visceral, and skeletal abnormalities were tabulated. Fetal weights were only recorded from animals at scheduled sacrifice. A scaled assessment of the manus and pes was also performed.

3. Plasma tyrosine and tissue enzyme activity analysis: Blood samples were pooled for all of the pups of each sex per litter. All blood samples were centrifuged, and the plasma was removed and stored at -20°C until analysis. Plasma tyrosine levels were measured by HPLC with UV detection using a Hichrom S5ODS2 column with a mobile phase gradient of 100% acetonitrile mixing with water:acetonitrile:trifluoroacetic acid (950:50:2; v/v). Livers and kidneys were weighed, homogenized, and centrifuged to yield cytosols that were then aliquoted and stored at -70°C until enzyme analysis was performed. Tyrosine aminotransferase (TAT) catalyzes the conversion of tyrosine to *p*-hydroxyphenylpyruvic acid (HPPA). HPPA will react with

phenazine methosulphate to form a colored reaction product that can be measured by a spectrophotometer at 675 nm. Cytosols were analyzed spectrophotometrically (in duplicate) for TAT activity. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) catalyzes the oxidative decarboxylation and rearrangement of HPPA to homogentisate, with incorporation of both atoms of molecular oxygen into the product. Thus, HPPD activity in solution can be measured by monitoring oxygen consumption. Cytosolic oxygen consumption was measured using an oxygen electrode.

D. DATA ANALYSIS: Data relating to animals which were not pregnant, died intercurrently, or totally resorbed or aborted their litters were excluded from statistical analysis. The following statistical tests were applied to the data. All tests were two-sided.

Parameter	Statistical test
Maternal body weights during dosing and post-dosing	Analysis of covariance (ANCOVA), with GD 8 body weight as the covariate followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test
Plasma tyrosine	Analysis of variance (ANOVA) followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test. Data were analyzed following a $\log_{10}$ transformation.
Maternal liver and kidney TAT and HPPD # of implantations # of live fetuses/female Gravid uterus weight Litter weight Mean fetal weight/litter (sexes combined and separate) Mean *manus* and *pes* scores	ANOVA followed, as necessary, by pair-wise comparison of treated groups with the control group via Student's t-test
Maternal performance data Proportion of fetuses with each individual *manus* and *pes* score Proportion of fetuses with each defect Proportion of litters with each defect	Fisher's Exact test
Pre- and post-implantation loss (%) Early and late intrauterine deaths Male fetuses Overall incidence of major or minor (only) external/visceral defects Overall incidence of major or minor (only) skeletal defects Overall incidence of skeletal variations	Percentages were analyzed by ANOVA following double arcsine transformation (Freeman and Tukey, 1950) Proportion of fetuses (except male fetuses) and proportion of litters affected were considered by Fisher's Exact test.

E. INDICES: The following indices were calculated from the cesarean records:

% Pre-implantation loss = (# of corpora lutea - # of implantations)/# of corpora lutea x 100
% Post-implantation loss = (# of implantations - # of live fetuses)/# of implantations x 100

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality: One control doe (#15) was found dead approximately 20 minutes post-dosing on GD 19. This pregnant animal had no previous signs of ill health, and did not demonstrate any signs of trauma due to dosing. There were no signs of dosing error found during necropsy, and the cause of death was not determined. One group 4 (500 mg/kg ZA1296 + 1% tyrosine) doe (#56) was killed on GD 22 following the abortion of several fetuses. This animal had demonstrated negligible food consumption from GD 17 and a loss of body weight from GD 19. Necropsy revealed a flaccid heart with pale areas on the ventricles; however, this finding was not considered to be treatment-related.

2. Clinical signs: Few feces were observed in 4/20 group 4 (500 mg/kg ZA1296 + 1% tyrosine) does compared to 1/20 controls; no feces were observed in 4/20 group 4 does compared to 0 controls. Dry sores on 1 or more area were observed in 4/20 group 4 does compared to 1/20 controls; and scabs on 1 or more area were observed in 4/20 group 4 does compared to 2/20 controls. However, these findings might not be associated with treatment. There were no other treatment-related clinical signs.

Table 2. Clinical signs of toxicity in the dams [#affected (#observations)] [a]

Clinical sign	Dose Group			
	0 mg/kg ZA1296 +0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
Few feces	1 (1)	0 (0)	1 (5)	4 (11)
No feces	0 (0)	0 (0)	2 (8)	4 (11)
Dry sores (1 or more area)	1 (7)	0 (0)	1 (7)	4 (26)
Scabs (1 or more area)	2 (18)	1 (5)	1 (17)	4 (49)

a Data were obtained from Table 2 on pages 41-42 in the study report.

3. Body weight: Maternal body weights and body weight gains are shown in Table 3. Maternal body weights (adjusted for initial weight) were decreased ($p \leq 0.05$) in the group 4 (500 mg/kg ZA1296 + 1% tyrosine) does during GD 14-21 (↓1-3%), and remained decreased (not significant) through GD 30 (↓2%). Overall (GD 4-30) body weight gains also were decreased in the group 4 does (↓12%) compared to controls. Body weights were also decreased ($p \leq 0.05$) in the group 2 (1% tyrosine only) does on GD 14 (↓1%), but this finding was considered minor and incidental. These differences in mean body weight and body weight gain are often seen in untreated rabbits and are not considered biologically significant at any dose level. No effects of treatment were observed on gravid uterus weights.

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Table 3. Maternal body weights and body weight gains (g)[a]

Gestation Day		Dose group			
		0 mg/kg ZA1296 0% tyrosine (n=16)	0 mg/kg ZA1296 1% tyrosine (n=14)	500 mg/kg ZA1296 0% tyrosine (n=18)	500 mg/kg ZA1296 1% tyrosine (n=17)
4[b]	Mean±SD	3463±293	3433±217	3417±193	3441±176
14	Mean	3602	3555* (↓1)	3577	3549* (↓1)
21	Mean	3717	3693	3662	3611* (↓3)
30	Mean	3886	3870	3864	3836 (↓2)
Overall gain (GD 4-30)	Mean[c]	433	412	411	381 (↓12)
Gravid uterus	Mean±SD	528±74	541±107	487±125	516±150

a Data obtained from pages 43-45 and 54 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.
b Adjusted means based on initial body weight
c Calculated by reviewers from the differences in unadjusted means on pages 43-45 of the study report.
* Significantly different from controls, $p \leq 0.05$

4. <u>Food consumption</u>: Maternal food consumption is shown in Table 4. Food consumption was decreased ($p \leq 0.05$) in the group 4 (500 mg/kg ZA1296 + 1% tyrosine) does by 27% on GD 8-11. Decreased food consumption continued throughout the remainder of the dosing period (GD 11-21), although without statistical significance (↓8-22%). Additionally, the group 3 (500 mg/kg ZA1296 only) does were observed to have decreased food consumption throughout dosing (GD 8-21; ↓7-13%; not significant). In both cases, food consumption returned to control levels by GD 24-27. No effect of treatment was noted on food consumption in the group 2 (1% tyrosine only) does. It was noted by the sponsor that a large number of food consumption values were affected by spillage, and were excluded from statistical analysis. Based upon the relatively small differences in the dosage groups (and high standard deviations) and the high spillage levels typically noted in rabbit studies, these differences do not constitute maternal toxicity. This is especially true since no clear effects upon body weight and gain were noted.

Table 4. Maternal food consumption (g/rabbit/day)[a]

Gestation Day	Dose group			
	0 mg/kg ZA1296 0% tyrosine n=14-16	0 mg/kg ZA1296 1% tyrosine n=5-13	500 mg/kg ZA1296 0% tyrosine n=15-17	500 mg/kg ZA1296 1% tyrosine n=12-17
Pre-dosing				
GD 4-8	168±43	169±24	156±36	165±30
Dosing				
GD 8-11	159±38	157±56	144±41 (↓9)	116±54* (↓27)
GD 11-14	160±31	168±20	140±37 (↓13)	148±56 (↓8)
GD 14-17	143±26	165±24	133±30 (↓7)	121±56 (↓15)
GD 17-21	160±28	176±26	145±46 (↓9)	125±67 (↓22)
Post-dosing				
GD 21-24	143±32	137±42	152±26	126±48 (↓12)
GD 24-27	120±22	108±28	120±40	118±25
GD 27-30	103±24	89±25	110±37	107±22

a Data obtained from page 46 of the study report. Percent differences from controls, calculated by reviewers, are *included in parentheses.*

* Significantly different from controls, $p \leq 0.05$

5. <u>Ophthalmoscopic examinations</u>: There were no effects of treatment observed during ophthalmoscopic examination.

6. <u>Gross Pathology</u>: There were no effects of treatment observed during macroscopic examination.

7. <u>Plasma tyrosine analysis</u>: Maternal plasma tyrosine levels are summarized in Table 5. The study clearly achieved the objective of manipulating maternal plasma tyrosine levels. Plasma tyrosine levels were increased ($p \leq 0.01$) in all groups in a step-wise fashion: group 2 (1% tyrosine only) does increased 65-188%; group 3 (500 mg/kg ZA1296 only) does increased 130-810%; and group 4 (500 mg/kg ZA1296 + 1% tyrosine) does increased 284-1773%. Maximum levels were observed in all groups at 12 hours after treatment with ZA1296, and returned to pre-dosing levels at 24 hours post-dosing. The reviewers noted that group 2 (1% tyrosine only) does demonstrated plasma tyrosine levels that peaked at the 12 hour sampling point, despite being gavaged with water only. This might be associated with a diurnal pattern of food consumption, as diet containing 1% tyrosine was available *ad libitum*.

Table 5. Maternal plasma tyrosine levels (nmol/mL; mean±SD)[a]

Day and time of sampling	Dose group			
	0 mg/kg ZA1296 0% tyrosine n=16	0 mg/kg ZA1296 1% tyrosine n=14	500 mg/kg ZA1296 0% tyrosine n=18	500 mg/kg ZA1296 1% tyrosine n=17
Day 8				
0 hr pre-dosing	84±16	75±10	81±15	79±12
12 hr post-dosing	69±13	199±37** (↑188)	560±217** (↑712)	1202±368** (↑1642)
24 hr post-dosing	77±14	90±32	177±92** (↑130)	414±348** (↑438)
Day 14				
0 hr pre-dosing	83±10	137±39** (↑65)	246±98** (↑196)	363±185** (↑337)
12 hr post-dosing	63±11	171±66** (↑171)	528±157 (↑738)	1077±334** (↑1610)
24 hr post-dosing	70±14	103±33** (↑47)	179±71** (↑156)	269±194** (↑284)
Day 20				
0 hr pre-dosing	57±17	112±54** (↑96)	171±100 (↑200)	338±246** (↑493)
12 hr post-dosing	51±12	136±31** (↑167)	464±165** (↑810)	955±317** (↑1773)
24 hr post-dosing	53±17	95±34** (↑79)	166±126** (↑213)	331±268** (↑525)
Day 29	53±11	57±8	67±13** (↑26)	66±9** (↑25)

a Data obtained from pages 47-48 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

** Significantly different from controls, $p \leq 0.01$

8. <u>TAT activity</u>: Maternal TAT activity in liver and kidney cytosols are summarized in Table 6. There was no effect of treatment on TAT activity in liver cytosols. In the controls, kidney TAT activity was approximately 4.5-fold lower than in the liver. Kidney TAT activity was decreased ($p \leq 0.01$) in the group 3 (500 mg/kg ZA1296 only) and group 4 (500 mg/kg ZA1296 + 1% tyrosine) does (↓42-58%) compared to controls.

Table 6. Maternal TAT activity (nmol HPPA/min/mg protein; mean±SD) in liver and kidney cytosols[a]

Organ	Dose group			
	0 mg/kg ZA1296 0% tyrosine n=16	0 mg/kg ZA1296 1% tyrosine n=14	500 mg/kg ZA1296 0% tyrosine n=18	500 mg/kg ZA1296 1% tyrosine n=17
Liver	3.82±0.85	3.89±0.97	3.77±1.27	3.72±0.91
Kidney	0.81±0.32	0.66±0.20	0.34±0.16** (↓58)	0.47±0.17** (↓42)

a Data obtained from page 50 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

** Significantly different from controls, $p \leq 0.01$

9. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) activity: Maternal HPPD activity in liver and kidney cytosols are summarized in Table 7. Liver HPPD activity was decreased ($p \leq 0.01$) in the group 3 (500 mg/kg ZA1296 only) and group 4 (500 mg/kg ZA1296 + 1% tyrosine) does (↓54-57%) compared to controls. In the controls, kidney HPPD activity was approximately 3.5-fold lower than in the liver. Kidney HPPD activity was decreased ($p \leq 0.01$) in the group 3 and 4 does (↓69-75%) compared to controls.

Table 7. *Maternal HPPD activity* (μL O_2/min/mg protein; mean±SD) in liver and kidney cytosols[a]

Organ	Dose group			
	0 mg/kg ZA1296 0% tyrosine *n=16*	0 mg/kg ZA1296 1% tyrosine *n=14*	500 mg/kg ZA1296 0% tyrosine *n=18*	500 mg/kg ZA1296 1% tyrosine *n=17*
Liver	1.90±0.25	1.94±0.39	0.81±0.36** (↓57)	0.87±0.29** (↓54)
Kidney	0.55±0.08	0.51±0.07	0.14±0.07** (↓75)	0.17±0.07** (↓69)

a Data obtained from page 49 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

** Significantly different from controls, $p \leq 0.01$

10. Cesarean section data: Cesarean section data are summarized in Table 8. One control and three group 2 (1% tyrosine only) does were found to have complete litter resorptions at necropsy; however, this effect was probably not related to tyrosine as no complete litter resorptions were observed in the other treated groups. No effects of treatment were observed on fetal body weight, numbers of litters, number of live fetuses per doe, resorptions (early or late), sex ratio, or post-implantation loss.

Table 8. Cesarean section observations[a]

Observation	Dose group			
	0 mg/kg ZA1296 0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
# Animals Assigned (Mated)	20	19[b]	20	20
# Animals Pregnant	18	17	18	18
Pregnancy Rate (%)[c]	90	89	90	90
# Nonpregnant	2	2	2	2
Maternal Wastage				
# Died	1	0	0	0
# Died Pregnant	1	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	1
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	182	178	186	188
Corpora Lutea/Dam	11.2±2.0	11.1±1.6	10.3±1.7	11.1±2.0
Total # Implantations	163	158	167	163
(Implantations/Dam)	10.0±1.7	9.9±2.2	9.3±2.2	9.6±2.8
Total # Litters	16	14	18	17
Total # Live Fetuses	142	125	147	145
(Live Fetuses/Dam)	8.9±1.6	8.7±1.9	8.2±2.5	8.5±3.2
Total # Dead Fetuses[d]	0	0	0	0
(Dead Fetuses/Dam)[c]	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Total # Resorptions	21	33	20	18
Early	16	24	13	8
Late	5	9	7	10
Resorptions/Dam	1.2±1.3	1.8±1.8	1.1±1.1	1.1±1.8
Early	0.9±1.0	1.3±1.9	0.7±1.0	0.5±1.2
Late	0.3±0.6	0.5±0.8	0.4±0.7	0.6±1.3
Litters with Total Resorptions	1	3	0	0
Mean Fetal Weight (g)	40.4±2.3	41.8±3.9	40.1±7.2	39.4±5.3
Males	41.2±3.2	41.9±4.1	39.8±9.2	39.7±6.1
Females	40.0±3.5	42.0±4.9	39.5±7.0	39.3±5.4
Sex Ratio (% Male)	48.9±18.8	50.1±16.8	44.4±18.2	39.7±18.2
Preimplantation Loss (%)	9.5±12.0	10.8±15.0	10.2±14.8	13.8±18.3
Postimplantation Loss (%)	10.7±11.4	11.8±11.2	12.6±13.9	11.4±17.5

a Data obtained from pages 40, 54-55, and 267-270 of the study report.

b One doe was excluded from the data as it was considered to have been mated one day earlier.

c Calculated by reviewers from data presented in this table

d The reviewers determined there were no dead fetuses because any post-implantation losses (i.e., differences between number of viable young and number of implantations) were entirely accounted for by the total number of resorptions.

B. DEVELOPMENTAL TOXICITY

1. External examination: External abnormalities are presented in Table 9a. Tail appearing as a flap of skin was noted in one group 2 (1% tyrosine only) fetus (0.8% fetuses; 7.1% litters) compared to 0 controls; although a rare finding , this finding might not be treatment related since it was only observed once and not observed in group 4 (500 mg/kg ZA1296 + 1% tyrosine). However, possible related effects were noted in group 4 including slightly kinked tail and shortened tail in a single fetus each (0.7% fetuses; 5.9% litters) compared to 0 controls. Slight forelimb flexion and missing claw were noted in a single control fetus each (0.7% fetuses; 6.3% litters). There were no other external abnormalities.

2. Visceral examination: Selected visceral abnormalities are presented in Table 9b. The following defects were observed in single group 4 (500 mg/kg ZA1296 + 1% tyrosine) fetuses (0.7% fetuses; 5.9% litters) compared to 0 controls: (i) omphalocoele; (ii) umbilical hernia; and (iii) internal hydrocephalus of the brain. Although historical control data were not presented for comparison, these defects were considered incidental and not related to treatment. Extreme constriction of the pulmonary artery was noted in the group 3 (500 mg/kg ZA1296 only) fetuses (0.7% fetuses; 5.6% litters) and group 4 fetuses (1.4% fetuses; 11.8% litters), and extreme dilation of the aorta was observed in the group 2 (1% tyrosine only) fetuses (0.8% fetuses; 7.1% litters), group 3 fetuses (1.4% fetuses; 11.1% litters) and group 4 fetuses (2.1% fetuses; 11.8% litters), both compared to 0 controls. Increased incidence ($p \leq 0.05$) of extra vessel(s) arising from the aortic arch was observed in the group 3 fetuses (4.8% fetuses; 27.8% litters) and group 4 fetuses (6.9% fetuses; 29.4% litters) compared to 0 controls. Enlarged ventricle and reduced ventricle of the heart were observed in the group 2 fetuses (0.8% fetuses; 7.1% litters) and group 4 fetuses (1.4% fetuses; 11.8% litters) compared to 0 controls. Slightly dilated ureter and misshapen spleen were observed in single group 4 fetuses (0.7% fetuses; 5.9% litters) compared to 0 controls.

3. Skeletal examination: Selected skeletal abnormalities are presented in Table 9c. Increased ($p \leq 0.05$) incidences of the following findings were noted: long thoracolumbar rib 13 in the group 2 (1% tyrosine only) fetuses (35.2% fetuses; 92.9% litters), group 3 (500 mg/kg ZA1296 only) fetuses (73.5% fetuses; 88.9% controls), and group 4 (500 mg/kg ZA1296 + 1% tyrosine) (92.4% fetuses; 100% litters) fetuses compared to controls (21.8% fetuses; 68.8% litters); and 27 pre-pelvic bilateral vertebrae in the group 3 (49.7% fetuses; 88.9% litters) and 4 (86.2% fetuses; 100% litters) compared to controls (14.1% fetuses; 68.8% litters). Increased ($p \leq 0.05$) incidence of the following findings were also noted: incomplete ossification of the odontoid of the cervical centra in the group 3 (13.6% fetuses; 38.9% litters) and group 4 (14.5% fetuses; 52.9% litters) fetuses compared to 0 controls; and incompletely ossified pubis in the group 4 fetuses (6.9% fetuses; 47.1% litters) compared to controls (0.7% fetuses; 6.3% litters).

The following malformations were observed in single group 3 fetuses (0.7% fetuses; 5.6% litters) compared to 0 controls: (i) interparietal bone integrated into supra-occipital; (ii) thoracic centrum 12 fused to arch 12; (iii) thoracic centrum 12 fused to centrum 11; (iv) thoracic centrum 12 hemicenter absent; (v) thoracic arch 12 absent; (vi) lumbar centrum 4 hemicenter absent; (vii)

lumbar vertebrae misaligned; (viii) sacral vertebrae arches widespread; (ix) caudal vertebrae arches widespread; and (x) thoracic rib 12 absent. The following major defects were observed in single group 2 fetuses (0.8% fetuses; 7.1% litters) fetuses compared to 0 controls: (i) sacral centrum 4 absent; (ii) sacral arch 4 absent; and (iii) caudal vertebrae absent. These defects were not observed in group 4.

4. Manus and pes assessment: Manus and pes assessment are presented in Table 9d. The proportion of animals having a manus score of 3 was decreased in the group 3 (500 mg/kg ZA1296 only; $p \leq 0.05$) and group 4 (500 mg/kg ZA1296 + 1% tyrosine; not significant) fetuses, with corresponding increases ($p \leq 0.05$) in the proportion of animals having a manus score of 4. Similarly, the proportion of animals having a pes score of 1 was decreased ($p \leq 0.05$) in the group 3 and 4 fetuses. with corresponding increases ($p \leq 0.05$) in the proportion of animals having a pes score of 2.

Table 9a. External abnormalities [% fetuses affected (% litters affected)][a]

Observations		Dose (mg/kg bw/day)			
		0 mg/kg ZA1296 0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
# Fetuses (# litters) examined		142 (16)	122 (14)	147 (18)	145 (17)
Tail:	flap of skin	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Tail: kinked	slightly	0 (0)	0 (0)	0 (0)	0.7 (5.9)
	shortened	0 (0)	0 (0)	0 (0)	0.7 (5.9)
Forelimb/paw:	slightly flexed	0.7 (6.3)	0 (0)	0 (0)	0 (0)
	missing claw	0.7 (6.3)	0 (0)	0 (0)	0 (0)

a Data obtained from page 60 in the study report.

Table 9b. Selected visceral abnormalities [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)			
	0 mg/kg ZA1296 0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
#Fetuses (# litters) examined	142 (16)	122 (14)	147 (18)	145 (17)
Abdomen				
omphalocoele	0 (0)	0 (0)	0 (0)	0.7 (5.9)
umbilical hernia	0 (0)	0 (0)	0 (0)	0.7 (5.9)
Brain				
internal hydrocephalus	0 (0)	0 (0)	0 (0)	0.7 (5.9)
Blood vessels				
pulmonary artery constricted (extreme)	0 (0)	0 (0)	0.7 (5.6)	1.4 (11.8)
aorta dilated (extreme)	0 (0)	0.8 (7.1)	1.4 (11.1)	2.1 (11.8)
extra vessel(s) arising from aortic arch	0 (0)	0.8 (7.1)	4.8* (27.8)*	6.9** (29.4)*
Heart				
ventricle enlarged	0 (0)	0.8 (7.1)	0 (0)	1.4 (11.8)
ventricle reduced	0 (0)	0.8 (7.1)	0 (0)	1.4 (11.8)
Ureter				
slightly dilated	0 (0)	0.8 (7.1)	0 (0)	0.7 (5.9)
Spleen				
misshapen	0 (0)	0 (0)	0 (0)	0.7 (5.9)

a Data obtained from pages 60-63 in the study report.
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$

Table 9c. Selected skeletal abnormalities [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)			
	0 mg/kg ZA1296 0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
#Fetuses (# litters) examined	142 (16)	122 (14)	147 (18)	145 (17)
Skull				
interparietal integrated into supra-occipital	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Thoracic centra				
centrum 12 fused to arch 12	0 (0)	0 (0)	0.7 (5.6)	0 (0)
centrum 12 fused to centrum 11	0 (0)	0 (0)	0.7 (5.6)	0 (0)
centrum 12 hemicenter - absent	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Thoracic arches				
arch 12 absent	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Thoracic ribs				
rib 12 absent	0(0)	0 (0)	0.7 (5.6)	0 (0)
Lumbar centra				
centrum 4 hemicenter - absent	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Lumbar vertebrae				
misaligned	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Sacral vertebrae				
arches widespread	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Sacral centra				
centrum 4 absent	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Sacral arches				
arch 4 absent	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Caudal vertebrae				
arches widespread	0 (0)	0 (0)	0.7 (5.6)	0 (0)
Caudal vertebrae				
absent	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Thoracolumbar ribs				
rib 13 - long length	21.8 (68.8)	35.2* (92.9)	73.5** (88.9)	92.4** (100)*
Pelvic girdle				
27 pre-pelvic vertebrae, bilateral	14.1 (68.8)	14.8 (57.1)	49.7** (88.9)	86.2** (100)*
Cervical centra				
odontoid incompletely ossified	0 (0)	0.8 (7.1)	13.6** (38.9)**	14.5** (52.9)**
Pelvic girdle				
pubis incompletely ossified	0.7 (6.3)	0 (0)	2.7 (16.7)	6.9* (47.1)*

a Data obtained from pages 64-73 in the study report.
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$

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Table 9d. Manus and pes assessment[%][a]

Observations	Dose (mg/kg bw/day)			
	0 mg/kg ZA1296 0% tyrosine	0 mg/kg ZA1296 1% tyrosine	500 mg/kg ZA1296 0% tyrosine	500 mg/kg ZA1296 1% tyrosine
Manus				
Score 1[b]	0.7	0.8	0.0	0.7
Score 2[c]	27.5	18.9	24.5	18.6
Score 3[d]	64.8	73.8	49.0**	56.6
Score 4[e]	5.6	6.6	21.8**	18.6**
Score 5[f]	1.4	0.0	4.8	5.5
Mean score/litter	2.80±0.32	2.86±0.30	2.99±0.58	3.15±0.70
Pes				
Score 1	93.7	96.7	86.4*	86.2*
Score 2	6.3	3.3	13.6*	13.8*
Mean score/litter	1.07±0.08	1.04±0.11	1.13±0.20	1.19±0.32

a Data obtained from page 74 in the study report.
b Metacarpals/metatarsals and the 1st, 2nd, and 3rd rows of phalanges fully ossified. (good)
c Metacarpals/metatarsals, 1st and 3rd rows of phalanges fully ossified; 2nd row of phalanges fully ossified with the exception of the 5th digit, which is partially ossified.
d One metacarpal/metatarsal partially ossified, remainder fully ossified; 1st, 2nd, and 3rd row of phalanges fully ossified with the exception of the 5th digit, 2nd row, which is partially ossified.
e One metacarpal/metatarsal partially ossified; remainder fully ossified; 1st and 3rd row of phalanges fully ossified; some of the 2nd row may be partially or not ossified.
f One metacarpal/metatarsal not ossified; remainder fully ossified; 2nd row of phalanges not ossified; occasionally phalanges in the 1st and 3rd rows not ossified; remainder partially ossified. (poor)

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: ZA1296 was not teratogenic but did produce minor and inconsistent changes in fetal ossification. However, there was good correlation between the incidence of specific changes in fetal ossification and plasma tyrosine levels, indicating a causal relationship with tyrosine rather than a direct effect of ZA1296. There was no evidence for an association with tyrosinaemia or treatment with 500 mg/kg ZA1296/kg/day and abortion in this study. Therefore, the possible association of ZA1296 and abortion in the rabbit has not been confirmed.

B. REVIEWER COMMENTS:

1. Maternal toxicity: There were no effects of treatment on maternal survival, clinical signs, ophthalmoscopic examination, or gross pathology. Maternal body weights (adjusted for initial weight) were decreased ($p \leq 0.05$) in the group 4 (500 mg/kg ZA1296 + 1% tyrosine) does during GD 14-21 but the difference was very small(↓1-3%), and remained decreased (not significant) through GD 30 (↓2%). Overall (GD 4-30) body weight gains were also decreased in the group 4 does (↓7%) compared to controls. However, in the rabbit both the small body weight and body weight gain changes observed in this study are considered minor, not biologically significant and

do not constitute maternal toxicity. As well, food consumption was decreased ($p \leq 0.05$) in the group 4 does by 27% on GD 8-11 and continued throughout the remainder of the dosing period (GD 11-21), although without statistical significance (↓8-22%). Additionally, the group 3 (500 mg/kg ZA1296 only) does were observed to have decreased food consumption throughout dosing (GD 8-21; ↓7-13%; not significant). In both cases, food consumption returned to control levels by GD 24-27. Rabbits are notorious for spillage of feed and differences like these in the absence of clear body weight changes are not considered biologically relevant.

Plasma tyrosine levels were increased ($p \leq 0.01$) in all groups in a step-wise fashion, with the greatest increases occurring in the group 4 does (↑284-1773%). Levels peaked in all groups at 12 hours after treatment with ZA1296, and returned to maintenance levels at 24 hours post-dosing. Kidney TAT activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓42-58%) compared to controls. Liver HPPD activity was decreased ($p \leq 0.01$) in the group 3 and group 4 does (↓54-57%). Kidney HPPD activity was decreased ($p \leq 0.01$) in the group 3 and 4 does (↓69-75%). No clinical signs or pathology were associated with these findings. Therefore, clear indications of maternal toxicity were not observed in this study.

2. **Developmental toxicity:**

a. Deaths/Resorptions: One group 4 doe was killed on GD 22 following the abortion of several fetuses. This animal had demonstrated negligible food consumption from GD 17 and a loss of body weight from GD 19. Necropsy revealed a flaccid heart with pale areas on the ventricles: however, this finding was not considered to be treatment-related. No effects of treatment were observed on numbers of live fetuses, resorptions (early or late) or post-implantation loss.

b. Altered Growth: No effects on fetal body weight were apparent. Increased ($p \leq 0.05$) incidences of the following skeletal defects l defects were noted: incomplete ossification of the odontoid of the cervical centra in groups 3 (13.6% fetuses; 38.9% litters) and 4 (14.5% fetuses; 52.9% litters), and incompletely ossified pubis in group 4 (6.9% fetuses; 47.1% litters). The proportion of animals having a manus score of 3 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a manus score of 4. Similarly, the proportion of animals having a pes score of 1 was decreased in groups 3 and 4, with corresponding increases in the proportion of animals having a pes score of 2

c. Structural alterations: Increased incidence ($p \leq 0.05$) of extra vessel(s) arising from the aortic arch was observed in groups 3 (4.8% fetuses; 27.8% litters) and 4 (6.9% fetuses; 29.4% litters). Enlarged ventricle and reduced ventricle of the heart were observed in group 2 (1% tyrosine only) fetuses (0.8% fetuses; 7.1% litters) and group 4 (1.4% fetuses; 11.8% litters) fetuses. Increased ($p \leq 0.05$) incidence of the following findings were observed: long thoracolumbar rib 13 in groups 2 (35.2% fetuses; 92.9% litters), 3 (73.5% fetuses; 88.9% controls), and 4 (92.4% fetuses; 100% litters), and 27 pre-pelvic bilateral vertebrae in groups 3 (49.7% fetuses; 88.9% litters) and 4 (86.2% fetuses; 100% litters). Extreme constriction of the pulmonary artery was noted in groups 3 (0.7% fetuses; 5.6% litters) and 4 (1.4% fetuses; 11.8% litters), and extreme dilation of the aorta was observed in groups 2 (0.8% fetuses; 7.1%

litters), 3 (1.4% fetuses; 11.1% litters), and 4 (2.1% fetuses; 11.8% litters).

This is not a guideline study and due to study design, no dose response assessment was possible; a NOAEL and LOAEL were not determined. **In conclusion, in the absence of maternal toxicity, developmental toxicity was observed in this study with ZA1296 (Mesotrione, 96.8% a.i.) at a dose level of 500mg/kg (gavage) with either 1% or 0% tyrosine mixed in the diet.**

This study is classified **acceptable/non-guideline**.

C. <u>STUDY DEFICIENCIES</u>: The following deficiency was noted, but does not affect the acceptability of this study, as the purpose was not to set a LOAEL:

- Historical control data were not provided by the investigators.

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Chemical: Mesotrione

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